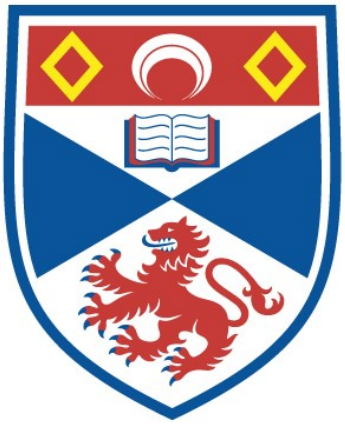
GENETICAL AND ENVIRONMENTAL STUDIES ON THE PROTEIN CONTENT OF WHITE HARICOT BEANS, PHASEOLUS VUIGARIS L., IN UGANDA

Yvonne Barbara Garrod

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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GENETICAL AND ENVIRONMENTAL STUDIES ON THE PROTEIN CONTENT OF WHITE HARICOT BEANS, PHASEOLUS VULGARIS L., IN UGANDA.

by

YVONNE BARBARA GARROD

A thesis submitted for the degree of Doctor of Philosophy to the University of St. Andrews.

Date:- 5th September 1975

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ABSTRACT

The present study has been initiated to investigate the genetical and environmental factors affecting the protein content of white haricot beans. A study of this nature requires the use of crude protein and methionine techniques to analyse large numbers of plant lines quickly and efficiently.

Two techniques are considered suitable for estimating percentage crude protein - the Kjeldahl-Markham technique for limited numbers of samples and the Automated nitrogen analyser for the large numbers of breeding samples. A microbiological method has been chosen for the analysis of available methionine.

These protein and methionine techniques are then used to assess the genetic variation in the collection of white haricot beans.

From this collection, twenty five varieties have been selected and assessed in Variety Trials over four different seasons to estimate the environmental effects on protein and methionine content. There is a considerable environmental effect on protein content and no effect on methionine content.

In the light of this environmental effect on protein content, the effect of different levels of fertiliser treatment are considered. The results show a significant variety x fertiliser treatment interaction for protein content, but not for methionine content. The protein-yield relationship has also been considered in relation to the increasing levels of fertiliser and the results show considerable variation according to the type of growth habit of the variety. The results indicate that for the protein inheritance study, it is important to grow all generations in one season and under as near to possible uniform environmental conditions.

A quantitative genetical approach to the study of protein inheritance has been adopted, using F_1, F_2 and F_3 generations of 7 x 7 non reciprocal diallel of selected contrasting parents. The genetic control of protein content has been studied using diallel cross analysis of Jinks and Hayman and the interpretation is based on the graphical presentation of the results of the analysis. The results suggest that protein content is controlled by a series of genes with minor effects which are additive but with partial dominance for low protein in at least some of the loci.

An assessment of the potential of the varieties has been made on the basis of their general and specific combining ability for protein.

The results obtained from the various aspects of the study are discussed in relation to the improvement of the bean crop.

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DECLARATION

I hereby state that my thesis entitled "Genetical and environmental studies on the protein content of white haricot beans, Phaseolus vulgarisL.,in Uganda", is not substantially the same as any that I have submitted for a degree or diploma or other qualification at any other University.

I further state that no part of my thesis has already or is being concurrently submitted for any such degree, diploma or other qualification.

Date:- 5th September 1995 Signed:-

STATEMENT BY THE AUTHOR

I hereby state that I was granted permission by the Senate of the University of St. Andrews on the 3rd July 1974 to submit my thesis entitled, "Genetical and environmental studies on the protein content of white haricot beans, *Phaseolus vulgaris* L., in Uganda", for a St. Andrews Ph.D. degree under Resolution of the University Court, 1967, No.1.

Date: 5th September 1995 Signed:

STATEMENT BY THE SUPERVISOR

I hereby state that the conditions of the Resolution and Regulations of St. Andrews University have been fulfilled. I have been a supervisor of the research project on which the thesis is based since September 1970. The student has been permitted under paragraph 3(b) page 29 of the handbook, "Higher study and Research 1974 - 75", to submit this work, carried out largely in Uganda from 1970 - 73 and subsequently at Cambridge. The special circumstances of this submission relate to the recent political circumstances in Uganda.

Date: 5th September 1975 Signed:

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LIST OF TABLES IN THE TEXT

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INTRODUCTION

Growing white haricot beans for canning, for export from East Africa, dates back to the 1930's. In 1937, a private company in Tanzania, Arusha Ltd., realised the possibilities of developing a canning bean industry in conjunction with seed bean growing, (Kuenzler, 1957). Because of the interest of these growers, production expanded rapidly after the war, as indicated by the following figures : 1949, 252 tons; 1950, 2029 tons; 1951, 2005 tons; 1952, 2553 tons, (Leakey, 1970 (a)). At this stage, a rapidly increasing number of inexperienced producers and middlemen entered the field and a lack of legislation resulted in a decline of crop quality. The 1953 exports were rejected almost entirely on account of weevil infestation. Further more, the beans then being grown for this trade were of mixed varieties, while the canners were becoming increasingly demanding about varietal characteristics and purity. Thus by 1954, although there was considerable interest in growing the crop, the market had become much more impenetrable. Production by 1957 had dropped to a mere 172 tons. Meanwhile in 1954-55, large supplies of mother seed of a Michigan Pea bean cultivar (probably Sanilac), internationally recognised as ideal for canning, had been introduced in an effort to keep the industry alive, (Macartney 1960, 1966). However, the cultivar was heavily attacked by rust, and this factor, together with poor yields, low prices in comparison with those in the seed bean trade, and the absence of a local market for the sale of rejected beans, virtually ended all further interest in the crop. The recovery of the white haricot bean industry in Tanzania has been slow but was occurring by 1966, (Macartney, 1966).

In 1966, world producers of canning beans were U.S.A., Canada, Australia and Japan and small quantities were exported by Holland, Ethiopia and Algeria, (Macartney, 1966). The U.S.A. and Canada now dominate exports. In East Africa, Kenya grows a limited supply of beans for canning within the country, but the product is generally not considered to be of highest quality.

In 1970 Uganda was offered a contract to grow canning beans for a major western company if its rigorous quality demands could be met. Since white haricot beans are not popular as food in Uganda, except in areas of Kigezi, Bunyoro and West Nile, the potential value of white haricot bean research would lie almost entirely in the prospects for economic production for export for canning, or for canning locally for an import substitute. For these purposes, very high quality standards and conformity to precise specifications are necessary. The canner requires a bean which is the right size, shape and colour, has the right flavour, cooks well, absorbs water readily, is split, crack and disease resistant and finally, interest was expressed by a well known buyer, in a bean of unusually high protein content. The seconds or low quality beans remaining that do not reach these specifications could be used on a large scale for animal feed, and possibly on a smaller scale for institutional feeding in the areas of Uganda mentioned above. For these secondary purposes a bean with a high methionine content is desirable since this is the first limiting amino acid in banana based diets.

Research on white haricot bean production in Uganda was conducted by Mukasa and Leakey at Kawanda, the Government Agricultural Research Station, during the period 1962-66

and led to the identification of Mexico 142 (sometimes known as Tangeru 16 or Kawanda 112) as the most suitable available cultivar from the agricultural and marketable viewpoint. However, it was far from ideal. Its yield potential is only three quarters that of some other white haricot beans already tested in Uganda, and it is susceptible to at least one race of Colletotrichum lindemuthianum (the pathogen causing anthracnose) and to East African Race D of Uromyces appendiculatus (the cause of bean rust), and its protein content is low.

By 1965 Uganda Government planners had received detailed advice from its Kawanda Research station for the development of the white haricot bean crop for export and there followed a meeting in June 1965 at which this development was agreed in principle. Both prison farmers and group farmers subsequently grew the crop on a large scale, but the necessary simultaneous provision of equipment for handling the crop for export never materialised, and a considerable embarrassment over large stocks of unwanted beans arose.

By 1967 stocks had been cleared or had become inviable and there was stated to be little seed available of Mexico 142 to supply prison farms, where interest in the potential of the crop still persisted.

Renewed interest in this crop at Makerere University arose as a result of Professor R. Holliday's particular interest in potential export crops and his desire that the O.D.A. Grain Legume Programme at Makerere should be strongly orientated in this direction. Happily, this coincided with enquiries in 1970

from a representative of H. J. Heinz Co. Ltd. for the possibility of production of 70,000 tons of white haricot beans for export to the U.K. as a pilot scale exercise with a guaranteed market at £70 per ton (the current price, 4 years later (Feb. 1974) had risen to £600 per ton!) for produce of suitable quality. The Uganda government indicated great interest in this potential development. Leakey (1970 (b)) prepared a proposal for Government detailing the necessary steps to achieve an export target of 70,000 tons of white haricot beans by 1972/73 on the basis of existing knowledge and the availability of one ton of certified seed of 'Mexico 142' beans by August 1970. The scheme had considerable but unavoidable capital implications involving, for example, the import of 70 Gunson Sortex 962 electronic colour sorting machines whose import needed to be phased from December 1970 onwards.

In subsequent discussion with Dr. Leakey, a representative of H. J. Heinz expressed an interest in the purchase of higher protein varieties of white haricot beans than were currently available. Leakey therefore initiated the present study to investigate the genetical and environmental factors affecting the protein content of white haricot beans. He had made a collection of a large number of white haricot beans at Kabanyolo, the University Farm. These consisted of a collection of 100 white haricot lines obtained from Tanzania; 3 commercial varieties received from Cambridge and 52 lines selected from the first 1300 entries of the World Collection of Beans, that combined white seed with tolerance or resistance to rust.

This collection of white haricot beans, together with lines developed at Kabanyolo by Leakey, provided the starting material for this study. The collection was assessed in detail, and

selections were made to initiate a hybridisation programme based on diallel crossing. The selection was based on all the commercially required characters but with particular reference to 'crude protein' content of the seed.

The programme of work was initiated with the objective of investigating:-

(1) the genetic variability of crude protein content in the collection of white haricot beans in Kabanyolo

(2) the environmental effect on crude protein content.

(3) the inheritance of crude protein content.

(4) the methionine content as a percentage of the crude protein content.

(5) the relationship between crude protein content and yield.

This required the use of crude protein and methionine techniques by which large numbers of plant lines could be screened quickly and efficiently. Investigations into crude protein and methionine techniques were therefore carried out. To do this, the literature on developments in protein and amino acid techniques was examined critically as a basis for choosing the techniques to be used for the analyses.

In the review of literature, it was also considered appropriate to examine the environmental and genetical studies on the protein content of the beans which had been carried out by other workers.

CHAPTER 1 REVIEW OF LITERATURE

1.1 Introduction

1.2 Developments in protein and amino acid techniques

1.21 Preparation of the sample for analysis

1.22 Protein - Nitrogen determinations

1.23 Hydrolysis of the sample for amino acid analysis

1.24 Recent methods of amino acid analyses

1.3 Environmental and genetical studies on the protein and methionine content

1.31 Introduction

1.32 Breeding for higher protein content

1.33 Breeding for amino acid balance

1.4 General Conclusion

1.1 Introduction

The plant breeder has been handicapped in the past by the lack of rapid, relatively inexpensive screening techniques for crude protein and specific amino acids. Selection in crop plants for high crude protein or high content of a specific essential amino acid, or both, has therefore not been widely practised in the past.

The review of literature therefore considers

(1) developments in crude protein and amino acid techniques and

(2) current environmental and genetical studies on protein content.

1.2 Developments in protein and amino acid techniques

The plant breeder needs techniques by which large numbers of plant lines may be screened quickly and efficiently for their protein content and nutritional quality. The degree of precision of the determination need not necessarily be very high but it must be precise within certain limits, while at the same time allowing very large numbers to be handled. For legumes, screening should involve the determination of total nitrogen content, and the content of those essential amino acids which are present in legumes in such low quantities to be likely to be limiting nutritionally. This includes methionine in particular. Such screening techniques must be based on sound analytical principles which imply low variability and good reproducibility. The cost per analysis, time per analysis and the ability for handling large numbers of samples need to be known as a basis for rational choice between alternative methods.

The scope of the operational problems involved in the analyses of proteins can be appreciated by considering the individual steps of this process.

The stages in the developments of protein and amino acid techniques are listed below.

1.21 Preparation of the sample for analysis.

1.22 Protein - Nitrogen determinations.

1.23 Hydrolysis of the sample, for amino acid analysis.

1.24 Recent methods of amino acid analyses.

The following review will discuss each step, cite various methods, and emphasise the currently preferred procedures, and the precision to be expected. Major emphasis will be devoted to quantitative procedures suitable for some particular essential amino acids found in proteins.

1.21 Preparation of the sample for analysis

The sample is usually ground and the moisture content obtained by drying the ground sample to a constant weight.

1.22 Protein - Nitrogen determinations

(1) Titrimetric

Nitrogen (as an estimation of crude protein) is most commonly determined by a micro-Kjeldahl procedure. Essentially the Kjeldahl method consists of a digestion, ammonia distillation and titration. The sample is digested for a few hours with boiling concentrated sulphuric acid and a catalyst, consisting of a mixture of $CuSo_4$, K_2SO_4 a Se. During this process, the nitrogen in the organic matter is reduced and retained in solution as NH_4^+ under acid conditions. After digestion, the ammonia ions are displaced and free NH_3 released by the addition of excess sodium hydroxide. Ammonia is distilled into an acid solution and determined by back titration with alkali.

There is extensive literature relating to the Kjeldahl method recorded in the Official Methods of Analysis of the Association of Official Agricultural Chemists (1933-1955). Accounts of numerous

modifications relating to type of catalyst, boiling temperatures, amounts of sample and chemicals etc. are recorded. An account of a recent modified Kjeldahl method is recorded in section 3.21 of Chapter 3.

Most of the modifications of the Kjeldahl method are concerned with increasing the speed of the analysis. Recently, Boyde (1971) increased the amount of potassium sulphate to raise the temperature by increasing the boiling point of the digestion mixture and thus considerably reduced the digestion time.

The traditional Kjeldahl method therefore has two disadvantages - it is a slow method and measures both protein nitrogen and non-protein nitrogen. Non protein nitrogen may consist of free amino acids, alkaloids, nucleic acids, amines, purines or pyramidines. Nevertheless, the Kjeldahl method is a reliable one in that it gives good reproducibility.

However, sophisticated, automated machinery has been developed by Technicon (Technicon Ltd., Chertsey, Surrey) to determine nitrogen. It is essentially a Kjeldahl digestion procedure followed by a colorimetric estimation of nitrogen. An average of sixty samples per day can be determined compared with twenty by the micro Kjeldahl method.

(2) Colorimetric

An example of a colorimetric method is the Dye-binding capacity method (DBC). It is based on the capacity of the basic groups of the constituent amino

acids (lysine, arginine and histidine) of proteins to combine with and precipitate a dye-molecule. In this method, an azo-sulphonic dye solution is mixed with a protein containing sample. The dye then bonds with the basic imidazol, guanidine and amino groups of proteins. These groups may originate from the basic amino acids, histidine, arginine and lysine as well as from free amino end groups of the protein chains (Frankel-Contrat & Cooper, 1944).

Mossberg (1971) pointed out that provided the fraction of the basic amino acids in the proteins is reasonably constant the correlation between dye-binding capacity and crude protein or nitrogen content would be sufficiently strong.

Until recently, this method has been used only sporadically to estimate the crude protein content in various foods. It has been used by Udy (1956) in wheat; in herbage by Outen et al (1966); soyabean by Pomeranz (1965) and milk and milk products by Ashworth et al (1960a and 1960b); meat by Torton and Whitaker (1964).

It would certainly be of great advantage to make routine determinations of DBC automatically. Preliminary experiments with Technicon continuous analysing system have been promising; it seems to be useful at least for filtering, colorimetric and registration processes. It also seems possible to combine the grinding and dyeing processes into one step, as in the Udy React-R-Mill, and thus automate almost the entire procedure.

The method seems to be well suited for plant breeding purposes as it offers a simple solution for the

screening of plant material for high basic amino acid content. Complementary analyses e.g. Kjeldahl for nitrogen and crude protein and chromatographic amino acid analyses for amino acid patterns, should then be used to determine whether the high content of basic amino acids in the types selected on the basis of DBC determinations is due to an increase in total protein content or to a change of amino acid pattern, of interest in either case. In this way, the use of more elaborate and expensive methods can be restricted to relatively few samples, while the rapid and inexpensive dye-binding method is used for mass screening.

(3) Physical

Physical and non destructive protein techniques have been prepared mainly by the IAEA (Luse, 1970 (a)). A co-ordinated programme has been established by the joint FAO/IAEA Division which is carrying out development evaluation and standardisation of methods for rapid mass screening of plant material (Luse, 1970 (b)). Lindegren (1971) described physical assays based on neutron activation of nitrogen which are being developed. The methods are not developed enough yet for practical purposes but when developed will be of great interest to the plant breeder because plant breeders have special interests in screening methods which allow non-destructive measurements of single seeds. Many of the methods permit simultaneous determinations of water content as well as several other compounds of agricultural interest.

1.23 Hydrolysis of the sample for amino acid analysis (to break the proteins down to smaller peptides and amino acids)

The nature of the hydrolytic conditions plays an important role if high precision is required in the analysis. It has recently been revealed (Boyde, private communication) that the lack of precision in hydrolysis is one of the greatest sources of error in estimating the amino acid contents of proteins. There are essentially three types of hydrolysis (i) acidic, (ii) alkalinic, (iii) enzymic. Acid hydrolysis is most widely used because it favours peptide bond cleavage. Alkalinic hydrolysis has to be restricted to special cases since it completely destroys some amino acids. Enzymic hydrolysis is also of limited value since the hydrolysis is often not complete and reproducibility may be lower than with acid hydrolysis.

(1) Acid Hydrolysis

Hydrochloric acid is most commonly chosen because of the faster rate of peptide bond cleavage compared to sulphuric acid. Also excess hydrochloric acid can be easily removed from the amino acid mixture by evaporation. It is customary to treat the protein with 2.5 to 5000 times its weight of 6N Hydrochloric acid and to reflux for 18-24 hours.

A serious problem with acid hydrolysis is the partial destruction of some amino acids. A substantial loss of tryptophane may occur and cysteine may be destroyed to a lesser extent.

(2) Alkaline Hydrolysis

Alkaline hydrolysis destroys arginine, serine, threonine and cystine and thus precludes the use of this approach for total amino acid analyses. Generally, the use of alkaline hydrolysis has been restricted to the study of amino acids that are acid labile. In particular, typtophane and tyrosine can be determined by alkaline hydrolysis.

(3) Enzymic hydrolysis

The availability of highly purified proteolytic enzymes suggests the use of enzymes for the complete hydrolysis of proteins. The known specificity of trypsin, chymotrypsin, papain, and pepsin for example, would indicate that any one of these could be used for hydrolysis. However, the hydrolysis may be incomplete and even the use of one enzyme followed by another often only achieves a 50-70% hydrolysis of the sample.

1.24 Recent methods of amino acid analyses

The prospects and limitation for rapid screening of large numbers of samples in a breeding programme are considered. Three important methods are discussed:

(1) Biological methods

(2) Chemical methods

(3) Physical methods

(1) Biological methods

These methods can be divided according to the organism used for the assay i.e. a) Protozoal methods

b) Bacterial methods

c) Use of higher animals

(a) Protozoal Methods

The classical studies of Kidder and Dewey (1957) on the amino acid requirement of the holotrich, Tetrahymena pyriformis, revealed the possibilities of protozoa as microbial tools for the study of protein quality. The potential of this animal micro organism lies in its ability to utilise intact protein, its absolute requirement for ten essential amino acids, its very high growth rate, and its propagation in pure culture on a chemically defined medium.

Initial attempts to develop T. pyriformis assays were by Rockland and Dunn (1949) using strain H, and they achieved a correlation with the results of nitrogen balance assays in rats. Further developments of assays using T. pyriformis strain W have been described by Pilcher and Williams (1954) and by Rosen and Fernell (1954, 1956). These workers used different methods of assessment of growth and computation of nutritive values. Pilcher and Williams (1954) modified the enzymic reduction of colourless soluble 2:3:5 - triphenyltetrazolium chloride to red insoluble triphenyl formazan by the organisms, for the assessment of growth response by haemocytometric count. They proposed that relative nutritive values be determined on the basis of number of ciliates grown per unit production of ammonia-nitrogen, ammonia being the end product of nitrogen metabolism. Inability to separate quantitatively the organisms from the residual food particles rules out the possibility of using dry weight, total nitrogen, protein nitrogen

or turbidity as measurements of growth or protein synthesis.

(b) Bacteriological Methods

These methods for amino acid analysis depend on knowledge of the nutritional requirements of bacteria and are successful with organisms having an absolute requirement for a particular amino acid to support normal growth. As assay consists of allowing bacteria to grow on a medium deficient in the amino acid under investigation and observing the rate of growth when graded amounts of the test protein are added. The test protein usually requires pretreatment with single or successive enzymes or chemical agents. This is because many of the bacteria are only partially proteolytic. The resultant hydrolysates serve as nitrogen sources for the organism. As little as 10 mg. sample of protein is required. Also the method is fairly sensitive and multiple analyses can be carried out in a relatively short time. Bacteriological methods are therefore being used widely for rapid screening of large numbers of samples in a breeding programme. However, there are limitations in using a bacterial assay. They are listed below:

(1) an inability of organism to use intact protein.

(2) their requirements for amino acids non-essential for higher animals.

(3) the probable influence of peptides stimulatory for bacteria, but not for higher animals (Kodicek and Mistry, 1957).

Some examples of the use of bacterial assays are discussed below.

Stokes et al (1945) describes a bacteriological method for the assay of ten essential amino acids, namely histidine, arginine, lysine, leucine, isoleucine, valine, methionine, threonine, tryptophane, and phenylalanine. The method is applicable to food stuffs and other natural products as well as purified proteins and synthetic amino acid mixtures. Nine of the amino acids are determined with Streptococcus faecalis and phenylalanine with Lactobacillus delbrukii C.D.5. Although two organisms were used, only one standard medium and one procedure was employed. The response of the two organisms to the amino acids was measured by titrating with alkali, the lactic acid produced during growth. A complete amino acid analysis could be carried out with 1.5 g. of sample. The method also yields many replicate results within a short time and is therefore useful for routine screening of large numbers of samples. The disadvantages of this method is the inability of the organism to use intact protein. Therefore the protein sample requires pretreatment with single or successive enzymes or chemical agents. The resultant hydrolysates serve as nitrogen sources for the organism.

Horn et al (1952, 1954) used Leuconostoc mesenteroides P-60 for evaluation of the protein in differently processed cotton-seed meals. They were digested on successive days with pepsin, trypsin, and pig mucosa and abilities of the enzymic hydrolysates to support growth of the organisms (acid production) were compared with responses on complete hydrolysates. The results agreed fairly well with rat growth tests, but the scope of the assay for other protein materials was not investigated.

Ford (1960) used a quick simple method in using a strain of Streptococcus zymogenes. This organism grows quickly with an adequately intact protein as the main source of nitrogen. It requires exogenous methionine, leucine, isoleucine, arginine, histidine, tryptophan and valine. Ford (1962) used Streptococcus zymogenes to measure the biological availability of these amino acids in a variety of food stuffs. The method involves a predigestion of the sample because S. zymogenes is only partially proteolytic. The sample is then inoculated with S. zymogenes and incubated overnight at 37°C before measuring the bacterial growth. The growth response can be measured by reading the optical densities of the cultures with a suitable photoelectric instrument or measuring the titratable acidities by titrating each culture with N/10 NaOH to an end point of pH 8.0, or by measuring the pH of each culture.

The above method outlined by Ford (1960) using S. zymogenes was modified by Boyne, Price, Rosen and Stott (1967). Further modification of these assay methods were carried out by Kelly et al (1970) to produce a method which could be applied readily to a large number of small samples of dry beans. This rapid available amino acid assay does not involve any predigestion of the sample, but relies entirely on the proteolytic action of S. zymogenes (Kelly et al 1970). He found that the correlation coefficient between his rapid method and Ford's (1960) papain digestion method for available methionine was 0.61 for 27 samples (significant at the 1% level). This is not a very good correlation indicating that Kelly's method is probably not very accurate - possibly because Kelly is relying totally on the proteolytic action of the bacteria when in fact it is only partially proteolytic. Also he suggests rough estimates of the samples and inoculum to be used. However, his method is being used widely amongst plant breeders (Silbernagal 1971, Lelegi et al 1972) because his method is rapid and therefore large numbers of samples can be screened quickly to pick out high or low methionine lines.

Sensitive microbiological plate assay methods depending essentially upon differences of amino acids from a hydrolysate have been developed by Bolinder (1968). The methods have been developed for the following eight amino acids : lysine, methionine, cystine, threonine, tryptophan,

tyrosine, arginine and histidine. The specificity and validity of each assay method has been thoroughly investigated. The mean value of the coefficient of variation of repeated assays is 8%. The general procedures for carrying out microbiological assays for amino acids on large plates (20 x 40 cm.) using the cup plate method, have been simplified to a large extent. A single basal medium, completely synthetic is used with minor changes for different amino acid assays, together with four strains of lactic acid bacteria as organisms. Furthermore, these plate methods require a small amount of sample (30 µl) for each amino acid assay, and employ a simple technique capable of doing large numbers of assays in a minimum of time. Finally, only simple inexpensive equipment is required for carrying out these assays. It is therefore probable that Bolinder's method will be used widely for rapidly analysing the large numbers of samples involved in a breeding programme.

(c) Use of higher animals

The ultimate proof of improved nutritional quality of a commodity is improved growth of the human or other animal that consumes it. Although chemical, physical or microbiological tests are used to indicate quantity or quality, laboratory animals or even humans must also be finally used as indicators of improvement of the nutrient value of the results of a plant breeding programme.

The accepted methods for measuring the nutritional value of proteins involving the use of common laboratory animals - mice, rats, chicks, dogs and others are not initially used since they are laborious and costly and much too slow for routine use of evaluating large numbers of protein samples. The methods are based on the estimation of protein efficiency ratio (P.E.R.). There are a few serious disadvantages of this method, namely (a) no allowance is made for maintenance requirements of the test animal (b) the result varies with food intake and (c) the assumption that gain in weight of the body is indicative of the protein tissue laid down is not always valid. However, a modification has been described by Bender and Doell (1956) whereby a control group of animals fed on a protein-free diet is included in the experiment, and the difference between the weights of this group and the test group is used in the calculation instead of merely weight gain. This procedure allows for maintenance requirements and also permits the evaluation of poor proteins which do not promote growth (and of which, consequently P.E.R. cannot be measured). The results are independant of food intake. The third criticism mentioned above is thought to be of minor significance under experimental conditions.

The use of higher animals is considered very important in the final analysis especially since nutritionists are aware that beans have a low

protein digestibility. At present information on this particular problem is limited, and it is not known whether these effects are caused by a more rapid movement of the cooked legume through the intestinal tract or by a resistance to protein hydrolysis by the gastro intestinal enzymes. In any case significant losses of nitrogen occur in faeces when beans are consumed. (Bressani et al 1963).

(2) Chemcial methods

Chemical assay methods will now be discussed but it must be remembered that they are of limited value for the evaluation of protein quality since biological availability is not regarded at all, and also complete hydrolysis of the sample must first be carried out. However, despite this, there is no doubt that they are very useful supplements to the biological methods.

A land mark in chemical methods for the analysis of amino acids was the complete resolution of the amino acids normally found in proteins (Moore and Stein, 1951). Spackman et al (1958) adapted the ion exchange system to automatic operation and simultaneous recording of the effluent curve. A simple outline of the method is as follows : A buffer is pumped through the column at a controlled and fixed rate, the column effluent is mixed with a regulated flow of ninhydrin reagent, and colour is developed by passage through a reaction coil maintained at 100^{o}. The steam from the reaction coil passes through a colorimeter; the photocell potential is plotted on an automatic recorder.

The product of the reaction of ninhydrin with amino acids has an absorption maximum of 570 mμ. A colorimeter operated at this wavelength is responsible for one of the traces on the recorder. The yellow colour resulting from the reaction of proline with ninhydrin necessitates the use of a second colorimeter at a wavelength of 440 mμ. The latter recorder is used solely for the calculation of the proline and hydroxyproline content. Finally, the last trace recorded is the absorption at 570mμ in a curvette of one third the normal width. The effect of a 1:3 dilution of the sample is obtained by this technique, permitting the calculation of concentrations of amino acids which are so high that they are normally off the scale. The operation of the system as a continuous flow technique and the protection of the effluent steam from the laboratory atmosphere results in a constant and stable base line which permits an increase in precision and sensitivity over the earlier methods. A recovery of $100 \pm 3\%$ for samples with the range of 0.1-3 μmoles/amino acid is readily obtained. A complete analysis of the protein hydrolysate is completed in less than 24 hours, and the procedure for regeneration of the columns permits a new analysis to be started each day.

The basic principle and design has been adapted and incorporated into several commercially available machines. Obviously this method cannot be used for mass screening. However it is a valuable method for accurate determinations of small samples while other

methods are being used for mass screening. A good example of this sort of situation is the use of DBC method which seems to be well suited for plant breeding purposes, offering a simple solution for screening for high basic amino acid content. Complementary analyses e.g. Kjeldahl analysis for nitrogen content and chromatographic amino acid analysis for basic amino acid pattern, should be used to determine whether the high content of basic amino acids in the types selected is due to an increase in total protein content or to a change in amino acid pattern. In this way, the use of the more elaborate and expensive methods can be restricted to the smallest possible amount of material, while the rapid and inexpensive dye-binding method - which can be automated - is used for mass screening. The dye binding method is described in section 1.22 Protein - Nitrogen Determinations.

Another chemical method developed for amino acid analysis is Gas-Liquid Chromatography (GLC). A recently improved GLC method is described by Darbre and Islam (1968). This procedure is based on the use of N-trifluoroacetyl amino acid methyl esters in place of the higher esters used previously. The separation of 23 amino acids can be carried out in 80-90 minutes. Inglis and Edman (1970) describe the difficulties of extending gas chromatography procedures suitable for pure proteins to such complex materials as legumes.

(3) Physical methods

Lindegren (1971) discusses the possibilities of using non-destructive screening techniques for

amino acids. He suggests the use of photoelectron spectroscopy. Photons of suitable energies eject photoelectrons from the electronic shells of the atoms. The binding energy of an electron might be calculated as the difference between the energy of the incident photon and the ejected electron. Photons can be produced with a well defined energy, and electron energies might be measured with a high degree of accuracy, therefore precise determinations of the binding energy is characteristic of the atom, and nitrogen may be detected. Theoretically, it may also be possible to characterise the chemical state and therefore get information about amino acid composition.

Four physical methods for mass screening for specific amino acids are being developed by the IAEA (Luse 1970 (a)).

(1) Double isotopic labelling and separation by thin layer chromatography of specific amino acids.

(2) Radio-isotopic assay of CO_2 from decarboxylase action on specific amino acids.

(3) Utilisation of ^{14}C labelled energy source in a microbiological assay of specific amino acids.

(4) Activation of plant nitrogen by MeV neutrons to measure total nitrogen.

1.3 Environmental and genetical studies on protein and methionine content

1.31 Introduction

Phaseolus beans can be considered sufficiently important enough in human diets to warrant substantial effort on genetic improvement, since in several parts of the world e.g. Africa, Central and South America, beans are being grown and used as a staple food commodity.

Research on genetic improvement of *Phaseolus* beans for nutritionally related factors is just in the beginning stage. However, some results of research in the field have been published and several excellent research programmes are in progress. However, most programmes have been rather superficial with little continued concentration of effort. Moreover, until very recently, initiation of breeding programmes has been limited because of the lack of suitable methods for screening germ plasm and breeding material for improved nutrition. To date, nearly all of the breeding work for nutritional factors has involved higher protein and better amino acid balance (Meiners and Litzenberger, 1970).

Breeding for higher protein in *Phaseolus* beans has not advanced much beyond the stage of screening to determine how much variability exists. Considerable veriability has been reported, but extremes may be due to adverse environmental conditions and this may be quite meaningless in terms of genetic improvement. The screening of

collections of germ plasm of beans by several workers has indicated that the significant variability in protein content may be due to either environmental or genetic factors.

1.32 Breeding for higher protein content

Silbernagel (1968) initiated work to screen all available bean material on the Udy Protein Analyser for total base amino acid content. He found a protein content variation from 17 - 30%. The ultimate objective of his programme is to improve both the quantity and quality of bean protein as a human dietry protein source. Current efforts are centred around establishing some of the environmentally influenced parameters that affect over-all protein content in beans, such as location, water stress, photoperiod, fertiliser etc. So far, Silbernagel (1969) has shown that it does not appear that the rates or types of nitrogen application has any effect on total protein content. Also no effects on protein content was observed due to differential rates of zinc used either as a seed fungicide treatment or as soil amendments at rates from 10 to 1600 lbs. per acre on normal soils. Phosphorus was not effective in altering the protein content. Likewise seed produced under differential water stress or excess did not appear to differ in protein content. However, differences as great as 6-7 per cent were observed in beans of the same varieties grown at different locations. Just what factors are involved is not known at this point, nor is it known whether

these differences were constant from season to season.

Silbernagel (1969) has also initiated a hybridisation programme involving crosses between high x high; high x low; and low x low protein lines to try to establish to what extent protein content is genetically controlled.

Rutger (1968) also initiated a programme to study the protein content of beans. He found a range of crude protein from 19-31% in 343 bean lines grown on a New York experimental station in 1967. Later, studies indicated that location and year of production greatly influenced crude protein content. Values of 24, 28, 34 and 37 per cent protein were obtained from four seed sources of the red kidney bean. However, in a limited study with eight varieties and two locations, there was no variety x location interaction for protein even though the separate variety and location effects were highly significant. Rutger (1971) is continuing with studies on the effect of environment on protein content and also looking at the effect of nodulation on protein content.

At Cambridge, Hamblin and Woolfe (1971) have initiated a programme to screen over three thousand lines of the world collection of beans. They are analysing for total nitrogen using a Technicon autoanalyser. To date, they have shown that there is no significant correlation between yield and protein content in Phaseolus vulgaris so they consider that there is a possibility of combining high yield with high protein in their material.

Leakey and Stabursvik (1970) began an investigation on the breeding of improved protein content and quality in dry seed beans in Uganda. They found that on the basis of food balance sheets, quite a lot of leguminous protein is regularly eaten and that methionine may be the limiting amino acid. They were therefore interested in the protein content and the percentage of methionine in the protein. They used the micro-Kjeldahl method for nitrogen determinations and an amino acid analyser for the estimation of methionine.

The review so far shows that breeding for higher protein has not advanced much beyond the stage of screening to determine how much variability exists. However, there is one report in the literature concerning protein inheritance.

Lele ji et al (1972) reported on the results of a study on bean protein inheritance and correlations between percent protein and seed yield in segregating generations. They selected five lines of dry beans (Phaseolus vulgaris L.) for differences in protein and they were used as parents in crosses. The F_1 F_2 and F_3 mean crude protein contents were generally between the parents, but slightly closer to the low-protein parent.

Broad sense heritability estimates ranged from 30.7% to 63.7%. The narrow sense heritability estimates were 20.1% for backcross and 5.0% and 12.0% based on F_3/F_2 regression. These low values indicate high environmental influence on crude protein content.

Yield and crude protein percentage were generally negatively correlated in F_2 and F_3 plants from crosses between low and high percent crude protein bean lines. Generally high yielding segregates tended to be relatively low in percentage crude protein. Among F_2 and F_3 progenies, however, there were plants that were high in seed yield and above average percent crude protein.

They used micro Kjeldahl analyses to determine total nitrogen and the percent crude protein was obtained by multiplying the nitrogen content by the factor of 6.25.

Work has also been carried out on the possible localisation of protein in the seed. Wood et al (1969) decided to characterise bean seed protein. They found that methionine and cystine occurred in very small quantities in seed protein and tended to vary with total protein content. They extracted proteins from various varieties grown in solution culture with radioactive sulphate and used discontinuous vertical polyacrylamide gel electrophoresis and separated them into 8-12 distinct bands. They suggested that sulphur containing amino acids may vary between the 12 bands that they located.

Juo and Strotzky (1970) used a similar though more sophisticated technique. Globulins, albumins, and basic proteins were extracted from the bean seeds and their distribution was in the ratio 3:2:1. The various fractions were analysed for total protein by the method of Lowry et al (1951) and subjected to

disc electrophoresis in 7.5 and 15% acrylamide gels essentially as described by Davis (1964) for soluble proteins using tri-glycine buffer (pH8.3) and by Reisfeld et al (1962) for basic proteins using β-alanine buffer (pH 4.5). Changes in the bands during germination indicated that stored proteins were present in each class. The globulins appeared to represent the major storage protein. The amino acid composition of the proteins were not studied.

There have been very few studies in the amino acid composition of proteins from legumes grown under different nutritional conditions. Khai and Pleshkov (1964) studied the effect of phosphate on the fractional distribution and amino acid composition of proteins from leaves and maturing pods of beans. The amino acid composition was determined by paper partition chromatography in four solvent systems. The results showed that the pods at both stages of development contained significantly less nitrogen than the leaves and especially less protein nitrogen. With a phosphorus poor legume there was a marked increase in non-protein nitrogen. The amino acid composition of proteins from leaves and maturing pods of bean plants were fairly stable and was only slightly affected by reducing the amount of phosphorus supplied.

Later Khai and Pleshkov (1964) looked at the effect of phosphorus and potassium on the amino acid composition of the bean seeds. They found that the seed protein fractioned into five groups : water soluble, 25% of N; proteins precipitated by dialysis against water for 36 hours, 22% of N; proteins soluble in 1M Kcl, 28% of N; proteins soluble in

75% ethanol, 3% of N; and proteins soluble in borate buffer at pH 10.0 with 0.2% sodium bisulphate, 22% of N. The crude protein content in the seeds varied appreciably depending on the conditions of nutrition, while the amino acid composition of the proteins was rather stable. The amino acid composition in each of the fractions was rather similar but with water soluble fraction being relatively tryptophan rich, the dialysis protein being lysine rich, solubles are rich in aspartic acid and serine, and the borate fraction rich in proline and glycine but poor in tryptophan and aspartic acid. Methionine was low in all fractions. But methionine is generally low in legumes (Bressani et al 1973).

1.33 Breeding for amino acid balance

In breeding Phaseolus beans for improved protein or better amino acid balance, research has progressed only to the point of examining the range of variability and determining if the level of amino acid content is genetically controlled. One reason for this may be the limited range of methionine content in lines examined so far. Another reason may have been the lack of a suitable method for screening large populations of breeding material for methionine content. In fact the lack of such methods has undoubtedly discouraged breeding for higher methionine and cystine content in all of the food legumes as well as other crops.

By modifying a microbiological assay for methionine, Kelly and coworkers (1971) at the Campbell Institute for Agricultural Research have developed a method suitable for screening breeding material. Using the microbiological assay method,

Kelly (1971) has screened 3,600 bean cultivars, lines and single plant selections grown at Rancocas, New Jersey. He selected 82 as having greater than 33% higher microbiologically available methionine in the mature seed than the Sanilac standard. Of these 63 again assayed more than 33% higher microbiologically in 1969. Kelly (1971) concluded that the level of methionine in mature seeds of the common bean is determined genetically and sufficient variation exists with the species to permit improvement through hybridisation and selection.

An item of critical concern is to know the genetical relationship between methionine content in the bean lines and their yielding ability, since in the final analysis, the latter remains the most important in the development of improved strains of any one crop.

More needs to be known about the variation in methionine content of other food legumes and the nature of its heritability. The work of Yohe et al (1972) with mungbeans indicates that in the 313 strains studied, methionine ranged from 0.55% to 1.78% of protein with a mean of 1.20%. Unfortunately the strains high in methionine have tended to be low in protein.

1.4 General Conclusion

It can be seen from the review that until recently plant breeding has been handicapped by the lack of rapid, accurate and relatively inexpensive screening techniques for protein and for specific essential amino acids. So that, identification and selection of plant materials for improved protein lines has been a slow expensive procedure. Only recently are true mass screening techniques for protein and amino acids becoming available to the plant breeder.

The Kjeldahl procedure for the determination of total nitrogen has been widely used though the method is not suitable for very large numbers of samples. However, the method has been automated so that it is labour saving and gives good reproducibility. Where the samples are too many for Kjeldahl analyses and the automated equipment is not available, the alternative sometimes has been to measure dye-binding capacity. Values obtained under particular conditions correlate fairly closely with Kjeldahl nitrogen, and a regression equation can be used to calculate predicted nitrogen. The variations from the regression arise from different concentrations of basic amino acids/g.N. The conversion factor used in all determinations is N x 6.25.

Up until now, microbiological assay has provided the only means of relatively large scale assay for methionine, and one has had to make the best of it. The advantages are that it requires no expensive specialised equipment. The disadvantages are that precision is relatively low and that, for people otherwise engaged in purely chemical work, a new type of procedure has to be mastered, and assays can fail inexplicably or give aberrant results.

Ion exchange chromatography is the present 'ideal' but materials require a preliminary oxidation so that methionine units are converted to the more stable 'sulphone' form. It is difficult to visualise the throughput from such analyses being adequate for a plant breeding programme, but it is valuable for checking and calibrating other procedures. Gas-liquid chromatography has been proposed but there are difficulties in the extension of procedures suitable for pure proteins (Inglis and Edman, 1970) to such complex materials as legumes.

The only published procedure, which, even from the claims of its opponents appear to meet the need for routine screening for methionine of large numbers of samples in a general analytical laboratory is that of Ussary and Gehke (1969). This is a colorimetric method using nitroprusside. The method involves a colour reaction with sodium nitroprusside on papain digests for use in automated equipment. Interference from histidine and typtophan is overcome by adding an excess of glycine. Both reproducibility and recovery of added methionine were reported to be satisfactory for a series of soyabean samples. Since then, more difficulty has been encountered in obtaining acceptable reproducibility, although the same procedure in non-automated form has been giving quite good results with cowpeas (Boulter, 1972).

So, the problem is to find a rational compromise between speed and precision. When deciding on the technique to be used, it is a matter of priority to check and compare the values of the particular technique with the results from ion exchange chromatography.

It has been shown that the environment has a considerable effect on protein content. It is to be expected that when the same variety has been grown under conditions that lead to the crop varying in protein content there will be a similar variation in the content of methionine (as a % of the protein). It remains to be determined how far methionine remains stable.

With regard to the inheritance of protein content, it has been shown in the literature review, that only one limited study has been reported. More knowledge of the inheritance of protein content is therefore desirable prior to initiating a breeding programme to raise protein levels since it is thought, that selection for very high protein in beans will be difficult because of a probable high environmental influence.

To summarise, it is clear that only recently are true mass screening techniques for crude protein and amino acids, becoming available to the plant breeder. This has resulted in a limited study on environmental and genetical factors affecting crude protein content and methionine content of dry beans, Phaseolus vulgaris. This present study considers some of these problems.

CHAPTER 2 DEDUCTIONS FROM THE LITERATURE REVIEW GIVING RISE TO THE OBJECTIVES OF THE INVESTIGATION

CHAPTER 2 DEDUCTIONS FROM THE LITERATURE REVIEW GIVING RISE TO THE OBJECTIVES OF THE INVESTIGATION

The conclusion of Chapter 1 shows that:-

1. Rapid mass screening techniques for analyses of crude protein and amino acids have only recently been developed to analyse protein quantity and quality satisfactorily in breeding programmes.

2. The environment has a considerable influence in the quantity of crude protein in the seed and there is limited information available on the influence of the environment on the quantity and quality of the protein.

3. The existing genetic variation in crude protein and protein composition of white haricot beans is not entirely known and there has also been a limited study of the inheritance of crude protein.

The investigation was therefore directed to:-

(1) investigate the available techniques for analyses of crude protein and methionine in order to select techniques appropriate to the samples and experimental equipment available.

(2) assess the magnitude of the environmental effect on the quantity and quality of the protein. This knowledge is essential to estimate efficiently the genetic variations in protein quantity and quality.

(3) assess the genetic variability of crude protein and amino acid composition of the beans.

(4) investigate the inheritance of crude protein content.

(5) assess the relationship between crude protein content and yield.

(6) investigate methionine content as a percentage of the crude protein.

CHAPTER 3 PROTEIN AND METHIONINE TECHNIQUES

3.1 Introduction

The assay of beans for their crude protein content and methionine content involves two separate problems:-

(i) the specification of a detailed analytical procedure that will allow a high throughput with sufficient precision and built-in checks against unexpected sources of trouble and

(ii) the choice of sampling procedures that will give results that have as much meaning as possible for the plant breeder.

During the initial stages of the investigation there were a limited number of samples and so the Kjeldahl digestion followed by Markham distillation procedure was adopted. The modified method which was used in this study is described in section 3.21 of this chapter, followed by a series of experiments to determine the degree of precision required and the errors involved in the technique.

It was hoped that later the nitrogen autoanalyser (essentially a Kjeldahl digestion technique followed by a colorimetric procedure) would be used to analyse quickly the large numbers of samples involved in the breeding programme. However, the machine was not available at Makerere. So, the samples were stored and analysed later on the nitrogen autoanalyser in the Department of Applied Biology, Cambridge. The techniques involved in the use of the autoanalyser are described in section 3.22 of this chapter.

With regard to the methionine analyses, the review of literature indicated that a microbiological method would be the obvious choice for the samples and equipment available since,

(1) no sophisticated or expensive equipment was required (important when working in a developing country!)

(2) the methods are rapid so large numbers of samples could be tested.

(3) the degree of precision appeared to be sufficiently good to pick out the low and high values.

Two methods were investigated - the methods outlined by Bolinder (1968) and Kelly et al (1970). Kelly's method was chosen for screening the samples and it is described in section 3.31. Since considerable time and effort was spent on the Bolinder method and it was thought to be a valuable technique if sufficiently pure amino acids are available, it is described in detail in the Appendix.

3.2 Protein techniques

3.21 Kjeldahl technique for estimating nitrogen

Total nitrogen is determined by the Kjeldahl digestion procedure followed by the Markham distillation. The method used is described below.

Materials and Methods

Reagents

Mixed catalyst : 160 g. anhydrous K_2SO_4

10 g. $CuSO_4 . 5H_2O$

3 g. Selenium powder

These were mixed with a mortar and stored in a dry container.

Sulphuric acid - concentrated 98% (N-free)

Sodium hydroxide - 50% W/V

Boric acid A.R. - 2% W/V

Mixed indicator : 0.099 g. bromocresol Green plus 0.066 g. methyl red in 100 ml. of ethanol

Hydrochloric acid - N/50 HCL

Method

There are three stages to the method (1) digestion (2) distillation (3) titration

Digestion

0.2 g. of the milled oven dry sample was weighed accurately into a clean dry 100 ml. Kjeldahl flask. 1.7 g. of the mixed catalyst was added and 10 ml. of concentrated 98% (N-free) sulphuric acid. The mixture was then digested until the contents turned a clear green. Digestion was then continued for a further 60 minutes. The

flask was then allowed to cool before adding about 15 ml. water. After cooling, the contents of the flask were transferred quantitatively to a 50 ml. volumetric flask, washing with cold distilled water. When cool the volumetric flask was made up to the mark and the contents mixed.

Distillation

10 ml. of the digest was pipetted into the nitrogen still (Markham type) with the addition of 10 ml. of 50% sodium hydroxide. The sample was steam distilled into 10 ml. of 2% boric acid containing 4 drops of the mixed indicator. The distillation was continued until the indicator turned green and over 30 ml. of solution remained in the conical flask.

Titration

The distillate was titrated with standard N/50 hydrochloric acid, the end point being reached when the indicator turned from green through grey to definite pink. A note was made of the ml. N/50 HCL required. Blanks on the reagent alone were also run at the same time. If the blank exceeded 0.05 ml. of acid one or more of the reagents contained an excessive amount of nitrogen or the apparatus was contaminated. The samples would then be re-run.

Calculation

$$\text{Nitrogen in the sample} = \frac{\text{ml. of N/50 HCL required} \times 0.4}{\text{weight of sample}}$$

The percentage crude protein was determined by multiplying the % nitrogen by the conversion factor 6.25.

All protein results using this method were determined on a dry weight basis.

A series of experiments set up to determine the degree of precision required and the errors involved in the Kjeldahl-Markham technique

Three varieties were used in each experiment. They were chosen because of their different growth habit forms since this may be important when choosing the seed sampling procedure.

The different habit forms are described briefly below. There are two main types the Determinate and the Indeterminate. Their characteristics are shown below.

Derminate	Indeterminate
1. Short bush type.	1. Long climbing type.
2. All flowers produced at approximately the same time i.e. concise development phase.	2. Considerable time period over which flowers are produced.
3. Short internodes.	3. Long internodes.
4. Little vegetative growth.	4. Considerable vegetative growth.
5. Early maturing.	5. Longer maturing.
6. Terminal flower bud.	6. No terminal flower bud.
7. Weak apical dominance.	7. Powerful apical dominance.

The indeterminate growth forms can also be divided into two types - the indeterminate bush type and the indeterminate vine type. The bush type has shorter internodes and weaker apical dominance; the vine type has longer internodes and powerful apical dominance.

The three growth habit forms considered are therefore the Determinate type, the Indeterminate bush type and the Indeterminate vine type. In the text these are referred to as D, IB and IV respectively.

The varieties used in this series of experiments were:-

Verdon D

NEP 2 IB

Mexico 184 IV

There were three replicates to each experiment.

The materials and methods for each experiment are described below followed by Tables 3.21.1 - 3.21.5 which summarise the results of each experiment. Detailed experimental results are shown in Appendix tables A3.21.1 - A3.21.7.

Experiment 1 Analytical Technique

Twenty five seeds from the early formed pods of a single plant were oven dried and milled. One sample was then weighed and digested. The digest was then analysed four times.

Experiment 2 Grinding Technique

Twenty five seeds from the first formed pods of a single plant were oven dried and milled. The sample was then divided into 5 sub-samples and a sample from each was weighed and analysed separately.

Experiment 3 Pod Sampling Technique

Three sets of samples from a single plant were analysed:-

(1) Twenty five seeds from the early formed pods,

(2) Twenty five seeds from the intermediate formed pods,

(3) Twenty five seeds from the late formed pods.

Each set of seeds were oven dried, milled and analysed separately.

Experiment 4 Single Plant Sampling Technique

Experiment 4(a)

Twenty five seeds from among the early formed pods, intermediate formed pods and late formed pods of a single plant were bulked. Three samples of this bulked seed were then oven dried, milled and analysed separately.

Experiment 4(b)

All the seeds from a single plant were bulked. Three samples were taken which were oven dried, milled and analysed separately.

Experiment 4(c)

Using the method of 4(a) for sampling the pods, a comparison was made between three single plants of the same genotype. Each sample was then oven dried, milled, and analysed separately.

Experiment 5 Bulk Sampling Technique

Twenty five seeds from selected stratified pods of 42 single plants of the same genotype were bulked. A sample was then oven dried, milled and analysed.

RESULTS

Table 3.21.1 Results of an investigation of the Kjeldahl-Markham technique showing % crude protein means ± standard errors (s.e.) and coefficient of variation values (C.V.) for Experiment 1 - . Analytical Technique

Verdon

	Mean of 4 ± s.e.	C.V.
Rep 1	27.4 ± 0.069	
Rep 2	28.9 ± 0.044	0.36
Rep 3	27.5 ± 0.044	

NEP 2

	Mean of 4 ± s.e.	C.V.
Rep 1	28.9 ± 0.019	
Rep 2	27.4 ± 0.124	0.42
Rep 3	27.7 ± 0.129	

Mexico 184

	Mean of 4 ± s.e.	C.V.
Rep 1	24.3 ± 0.049	
Rep 2	30.3 ± 0.279	0.82
Rep 3	26.8 ± 0.036	

Table 3.21.2 Results of an investigation of the Kjeldahl-Markham technique showing % crude protein means ± standard errors (s.e.) and coefficient of variation values (C.V.) for Experiment 2 - Grinding Technique

Verdon

	Mean of 5 ± s.e.	C.V.
Rep 1	25.3 ± 0.039	0.36
Rep 2	26.9 ± 0.044	
Rep 3	26.3 ± 0.062	

NEP 2

	Mean of 5 ± s.e.	C.V.
Rep 1	29.3 ± 0.029	0.63
Rep 2	27.2 ± 0.039	
Rep 3	23.3 ± 0.119	

Mexico 184

	Mean of 5 ± s.e.	C.V.
Rep 1	28.9 ± 0.029	0.38
Rep 2	23.6 ± 0.105	
Rep 3	25.3 ± 0.039	

Table 3.21.3 Results of an investigation of the Kjeldahl-Markham technique showing % crude protein values and coefficient of variation values (C.V.) for Experiment 3 - Pod Sampling Technique

Verdon

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
(3) 29.5		(3) 28.0		(3) 22.7	
(2) 26.9	5.00	(2) 30.1	5.10	(2) 24.5	15.44
(1) 27.5		(1) -		(1) 30.3	

NEP 2

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
(3) 30.1		(3) 25.9		(3) 24.1	
(2) 27.2	5.22	(2) 27.2	6.18	(2) 27.9	9.44
(1) 29.4		(1) 29.2		(1) 29.0	

Mexico 184

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
(3) 24.1		(3) 25.2		(3) 28.7	
(2) 24.8	2.48	(2) 28.6	9.77	(2) 31.8	6.82
(1) 25.3		(1) 30.6		(1) 32.7	

Key (1) early formed seeds. (2) intermediate formed seeds
(3) late formed seeds

Table 3.21.4 Results of an investigation of the Kjeldahl-Markham technique showing % crude protein means ± standard errors (s.e.) and coefficient of variation values (C.V.) for Experiment 4 - Single Plant Sampling Technique

Experiment 4(a)

Verdon

	Mean of 3 ± s.e.	C.V.
Rep 1	26.3 ± 0.064	0.66
Rep 2	27.3 ± 0.065	
Rep 3	27.2 ± 0.182	

NEP 2

	Mean of 3 ± s.e.	C.V.
Rep 1	28.8 ± 0.042	0.84
Rep 2	27.3 ± 0.246	
Rep 3	27.7 ± 0.117	

Mexico 184

	Mean of 3 ± s.e.	C.V.
Rep 1	25.3 ± 0.104	0.76
Rep 2	26.5 ± 0.156	
Rep 3	28.8 ± 0.091	

Table 3.21.4 (contd.)

Experiment (b)

Verdon

	Mean of 3 ± s.e.	C.V.
Rep 1	25.6 ± 0.333	
Rep 2	26.7 ± 0.485	3.66
Rep 3	26.2 ± 0.675	

NEP 2

	Mean of 3 ± s.e.	C.V.
Rep 1	27.3 ± 0.605	
Rep 2	25.6 ± 0.140	5.27
Rep 3	25.9 ± 1.215	

Mexico 184

	Mean of 3 ± s.e.	C.V.
Rep 1	27.4 ± 0.919	
Rep 2	26.3 ± 0.062	4.48
Rep 3	27.2 ± 0.737	

Table 3.21.4 (contd.)

Experiment (c)

Verdon

	Mean of 3 ± s.e.	C.V.
Rep 1	26.1 ± 0.389	
Rep 2	26.8 ± 0.258	1.68
Rep 3	27.4 ± 0.127	

NEP 2

	Mean of 3 ± s.e.	C.V.
Rep 1	27.9 ± 0.437	
Rep 2	27.9 ± 0.486	3.19
Rep 3	27.7 ± 0.617	

Mexico 184

	Mean of 3 ± s.e.	C.V.
Rep 1	25.4 ± 0.545	
Rep 2	26.5 ± 0.583	3.90
Rep 3	27.2 ± 0.655	

Table 3.21.5 Results of an investigation of the Kjeldahl--Markham technique showing % crude protein means ± standard errors (s.e.) and coefficient of variation values (C.V.) for Experiment 5 - Bulk Sampling Technique

	Mean of 3 ± s.e.	C.V.
Verdon	27.5 ± 0.378	2.91
NEP 2	27.6 ± 0.991	4.39
Mexico 184	27.2 ± 0.328	2.09

Discussion and conclusion

A knowledge of a truly representative figure for the crude protein content of a variety can only be gained by use of a suitable sampling procedure in the field combined with a rapid but accurate chemical screening method in the laboratory.

The micro-Kjeldahl method for determination of crude protein (N x 6.25) was adopted after the procedure had been shown to operate satisfactorily on a routine basis.

The variation in the Analytical Technique and Grinding Technique of Experiments 1 and 2 (see Tables 3.21.1 and 3.21.2) was negligible.

The next step was to determine how plants in the field should be sampled and from the field sample, how many beans should be selected for chemical analysis. Since the protein content of single plants was of interest for future experiments, Experiment 3 considered the variation of protein content within a single plant. Details of the experimental results can be seen in Appendix Table A3.21.3.The results showed that the seeds in the early formed pods had a high protein content, the seeds in the intermediate formed pods had a lower protein and the seeds in the later formed pods had an even lower protein content. This effect was demonstrated in each of the three plant types though it was less marked for the determinate variety, Verdon. This could be explained by the fact that the seeds in the pods formed early in the plant life were filled when protein synthesis was active in

the plant; while later in the plant life when protein synthesis was not so active, the later formed pods were filled. These results indicate that positions over the entire plant would require harvest to give a representative sample. It would certainly not be representative to collect the top pods only i.e. those within easiest reach.

The results from Experiment 4(a) where 25 seeds from the three levels were harvested and bulked, show that the variation has decreased compared to Experiment 4(b) where 25 seeds from among the plant were harvested randomly.

The results from Experiment 4(c) showing the variation between single plants are less clear. The variation between single plants could be the result of any number of environmental factors. For example, a seed germinating later than its neighbours may be affected by the time of rainfall, nitrogen availability, spread of disease etc. Any one of these factors could affect the rate of protein synthesis at the time of pod setting and filling of the seed.

Thus, the results indicate that when harvesting a single plant for protein analysis, a selection of 25 seeds from the three levels of the plant would give a representative sample with a minimum of variation.

The results of Experiment 5 (see Table 3.21.5) indicate the kind of variation expected if the entire crop of mature beans were required for harvesting.

The human error was also considered. This could include (1) weighing the sample (2) measuring volumes of solution (3) reading the burette.

To avoid error in weighing the sample, a sample of approximately 0.2gm. was weighed and recorded accurately. When measuring volumes of solutions, particularly the sulphuric acid for digestion, the sodium hydroxide for distillation, and the boric acid for titration, it was important to use excess rather than less of the stated amount. With regard to the burette reading, it was important to read the burette to the nearest 0.04 ml. since the average volume of a drop from the burette was 0.04 ml., thus limiting the degree of precision to this amount. A difference of 0.04 ml. in the burette reading gives a difference of 0.2% protein after calculation. All samples were therefore run in duplicate and rejected if the two readings were not within 0.04 ml. of each other.

3.22 Automated analyser for estimating nitrogen

Materials and Methods

Reagents for (a) digestion

Kjeltabs (auto). One tablet contains 1.5g. K_2SO_4+ Selenium powder

Mixed acids 95% H_2SO_4 (N-free)
5% H_3PO_4 (88%)

Hydrogen peroxide (100 volumes)

for (b) the autoanalyser

Sodium hypochlorite, 50 : 50 hypochlorite : water.

Sodium phenate - 500 ml. of 40% NaOH was added slowly with cooling to 450 ml. of 80% phenol. A few drops of wetting agent were also added and the solution made up to 1500 ml. with water. This reagent was prepared fresh each day.

Wash solution - 50 ml. of mixed acids were diluted to 1000 ml.

Method

(a) Digestion

All samples comprising 25 seeds from the 3 stratified positions on a single plant were finely ground. A sample containing about 5 mg. of nitrogen was weighed out into a small paper cup on a torsion balance. For beans, this is up to 250 mg. wet weight. Each sample was

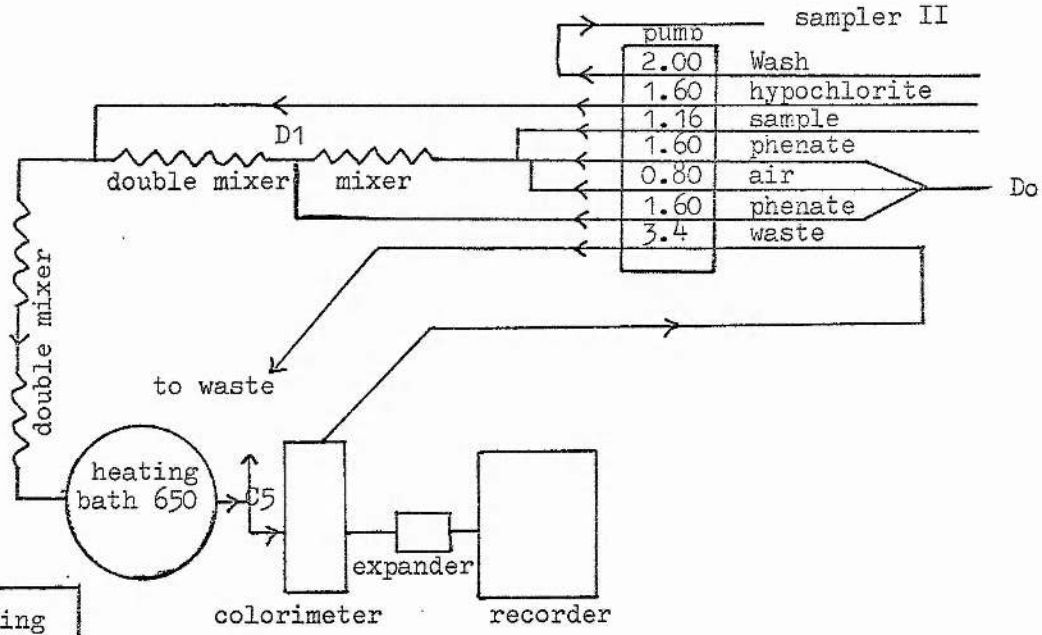
weighed out in duplicate. A block of 50 cups was then dried overnight at 105°. Each sample was then reweighed after cooling and dropped into a Kjeldahl flask (graduated up to 150ml.). One Kjeltab (auto) and 10ml. of the mixed acid were added. Also 3ml. of hydrogen peroxide were added slowly. When effervescence had subsided the sample was digested rapidly on an electrothermal unit at 600° until the solution was clear (approximately half an hour). Digestion was then continued for a further half an hour. After the digest had cooled, approximately 150 ml. of distilled water was added and the digest allowed to cool overnight. The sample was then made up to 150ml. This solution was then used for the next stage of the analysis.

(b) Autoanalysis

A diagram of the autoanalysis equipment is shown in Fig. 3.22.1.

The machine was switched on at the mains and the water bath brought to a temperature of 65°. The colorimeter was also switched on in advance. The machine was cleaned by passing decon through all the tubes for approximately one hour. The reagents were then run through the machine for 15 minutes, and a bubble pattern was established in the mixing coil. In the meantime the cups in the trays were filled. Five cups were filled with five known solutions of phenacetin (7.82% nitrogen) for the standards.

Fig. 3.22.1 A plan of the Nitrogen Autoanalyser



Normal flow	Internal diameter	Colour coding
2.00	0.073	Green
1.60	0.065	Blue
1.16	0.020	Yellow-orange
0.80	0.045	Red
3.40	0.100	Purple-orange

Also one cup for alanine (of known nitrogen content) and two cups for acid washes. The remainder of the cups were filled with sample solutions. One such tray of cups took approximately one hour and twenty five minutes to be analysed.

(c) Recording

The peak heights of the five standards, alanine and the samples were measured in mm. from the recording sheet.

(d) Calculation

The calculation was programmed for a Sharp calculator machine. The slope of the line for the standards was first determined from the graph of % nitrogen in standards against the peak heights in mm. of the standards from the recording sheet. This was then checked by determining the coefficient of variation to see how close the fit of the points was to the line. The slope of the line was then multiplied by the constant 6.25 and then by the value $\frac{\text{peak height of sample in mm.}}{\text{weight of sample}}$ for each sample to give the % crude protein content.

(e) Acceptability of Results

The results were only accepted if:-

(1) the phenacetin standards gave a linear response.

(2) for each individual sample, the replicates were within 1.0 percentage units of each other.

The method was generally satisfactory though from time to time gross aberations could occur. These could have been due to blockages in the

Analyser system, contamination of sample cups. or to 'operator' errors e.g. in writing down weights. Although drying introduced an extra step, it did seem important to express all results on a dry weight basis.

Experiments to determine the degree of precision required and the errors involved in the use of the Nitrogen Autoanalyser

The Department of Applied Biology, Cambridge carried out a series of experiments to determine the degree of precision required and the errors involved in the N-Autoanalysis technique. Therefore, it was unnecessary to repeat the procedure. The experiments carried out, by J.A. Woolfe and J. Hamblin in 1971 are listed below:-

(i) Six repeated runs (three on each of two successive days) on the same digest of each of twenty different bean varieties on the Technicon autoanalyser. A wide range of protein content, 22-33% existed amongst these 20 varieties.

(ii) Repeated digestions (seven in all), each in duplicate of of one grinding of 10 beans of the variety Provider (V1630)

The errors found in the analytical procedure both for the digestion and automated analysis steps, were considered sufficiently small for the procedure to be adopted as planned.

The standard deviation of the mean for six runs through the autoanalyser of the same digest was on average 0.36 with a range for the 20 digests from 0.22 to 0.57.

The mean % crude protein content found from 7 repeated duplicate digestions (4 digests) of 1 grinding was 27.5, standard deviation $\pm$ 0.49. Details of the experimental results are shown in the Appendix Tables A3.22.1 and A3.22.2.

3.2 3 General Discussion and Conclusion

A sample of 25 seeds from the early, intermediate and the late formed pods from a single plant, was the procedure adopted for both analytical techniques.

When analysing a limited number of samples, the Kjeldahl-Markham procedure was adopted. The Technicon Autoanalyser system was used when large numbers of samples were to be analysed since it was labour saving and gave good replication. However, both techniques have one disadvantage in that they both measure protein nitrogen, and non protein nitrogen.

3.3 Methionine techniques

Two methods were investigated - the methods outlined by Bolinder (1968) and Kelly et al. (1970). The aim was to find which technique was most suitable to screening the large numbers of samples from the breeding programme. The technique must obviously be rapid and reliable. It need not necessarily be highly precise so long as it picks out the potentially significant differences in the methionine levels among the bean lines.

Difficulties arose with the Bolinder method because the amino acids of the medium were not of sufficient purity. An account of the Bolinder method is recorded in the Appendix to section 3.3 of Chapter 3 of the Appendix. The Kelly method was therefore chosen since it gave reasonable consistency and sufficient quantitative accuracy. An account of the materials and methods of the Kelly method and a discussion of its suitability for this investigation are discussed in the following sections - section 3.31 and 3.32.

3.31 Kelly's method

This method determined available methionine. No predigestion of the sample was required because the method relied entirely on the proteolytic action of Streptococcus zymogenes NCDO 592. Ten milligrams of dried bean meal was placed in a culture tube and the sample was ready for assay.

The assay procedure was as follows. Eight milliletres of water and three milliletres of methionine - free assay medium (for details of the assay medium see Appendix Table A3.31.1) were added

to each tube. The tubes were covered with plastic caps drilled with 1mm. holes. The tube racks were covered with aluminium foil and autoclaved for twenty minutes at 100°C. Inoculum prepared and maintained according to the procedure outlined by Boyne, Price, Rosen and Stott (1967), (for details see Appendix Table A3.31.2), was drawn into a sterile 2cc. syringe fitted with a $1\frac{1}{2}$ inch twenty guage hypodermic needle. The aluminium foil was temporarily lifted and one crop of inoculum was added to each tube. Control tubes were left uninoculated. These served as standards to correct for turbidity from the bean cotyledons. The foil lid was replaced and the racks were incubated 24 hours at 37°C. The cultures were then killed by steaming for ten minutes in the autoclave, stirred, and the turbidity was read within 5 minutes (to permit seed coats and other undigested particles to settle) on a colorimeter set at a wavelength of 580 mμ. An alternative method, of reading the pH was also used.

Tubes containing 0, 5, 10, 15, 20 and 25 micrograms of pure methionine were tested using the same method as the samples and used to produce a standard curve for each batch of inoculum used. The results were calculated by plotting a curve of the average response to the standard and then reading off the test samples. Results were expressed as mg. methionine/g.dry bean.

A sampling experiment to determine the precision of Kelly's available methionine assay

Materials and Methods

The analytical procedure was carried out as described in the previous section.

For setting up the standard graph, optical density determinations were first used as shown in Fig. 3.31.1. However, the method was slow and laborious and it was thought that maybe particles of seed coat and undigested particles would interfere in the turbidity measurements. pH determinations were therefore used. This method was quick and gave good results. Only occasionally did the standard graph tail off at higher concentrations of methionine (as shown in Fig. 3.31.2), possibly indicating that the quantity of methionine was at saturation point for the organism at that time. A typical standard graph using pH measurements is shown in Fig. 3.31.3.

The seven varieties for the breeding programme were chosen as the test varieties. The varieties were : Mexico 142, Sanilac, F.M.52, Kabacuara, Pop412, W.C. 1210, and NEP2. They were chosen because they showed wide differences in protein content and other characters. Two samples of each variety were tested using three different inocula batches. The analytical method as described at the beginning of this section was used for each sample. The test samples were analysed using pH measurements and then read off against the standard graph. The results and the analyses of variance are shown in Table 3.31.1.

Fig. 3.31.1 A standard graph showing optical density values for six levels of methionine

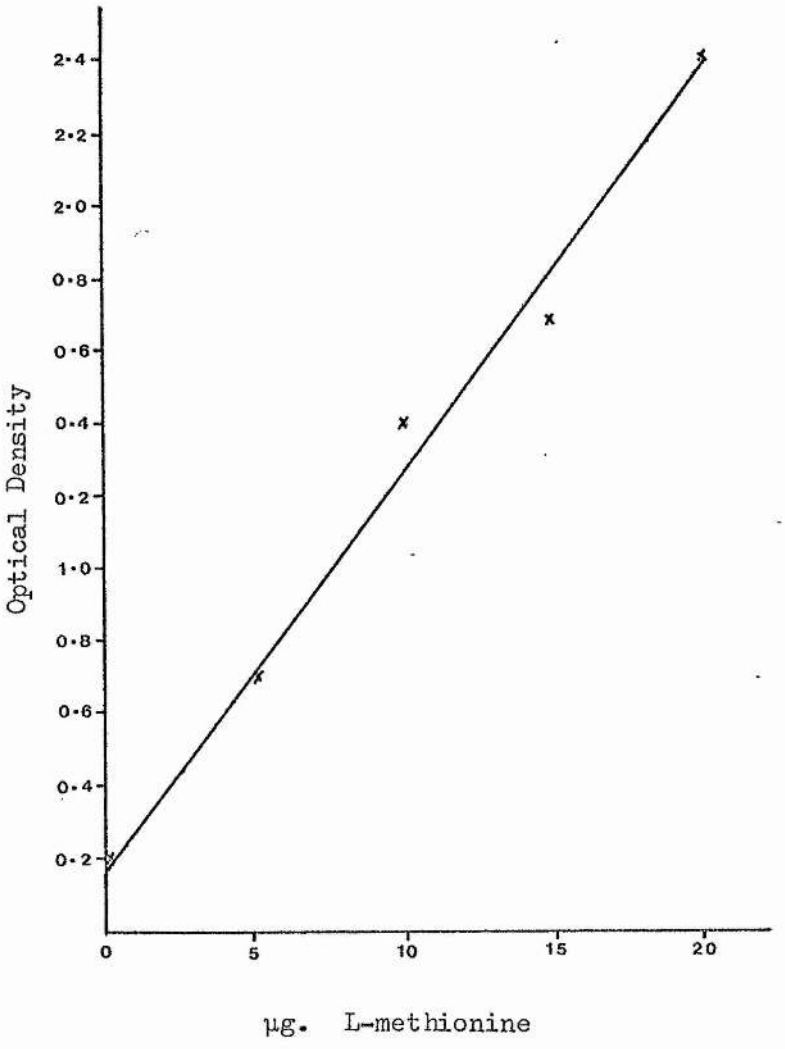


Fig. 3.31.2 Standard graph showing pH values for six levels of methionine

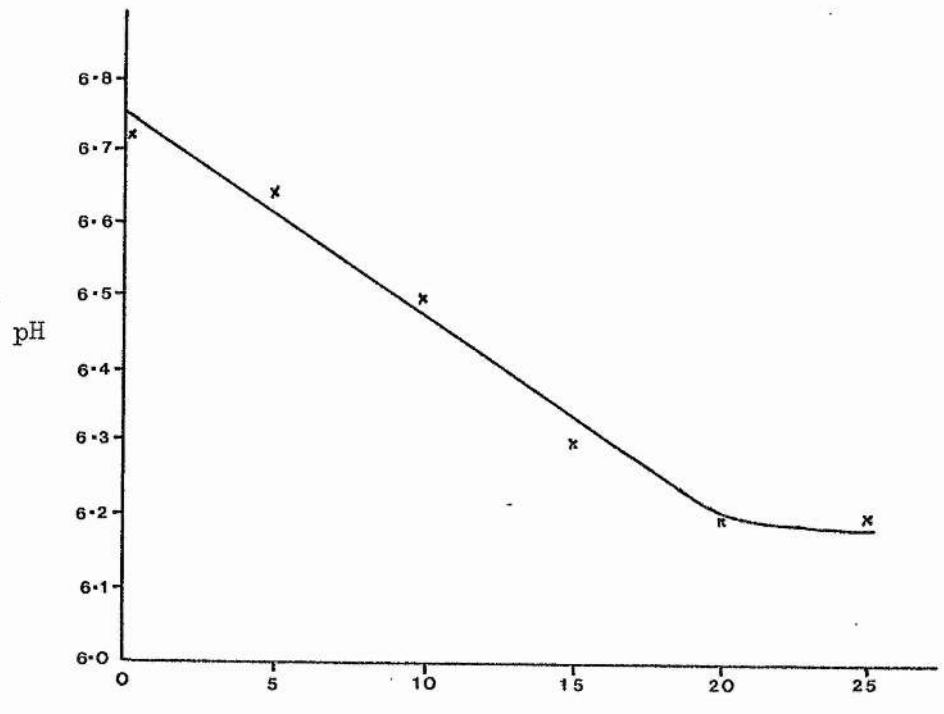
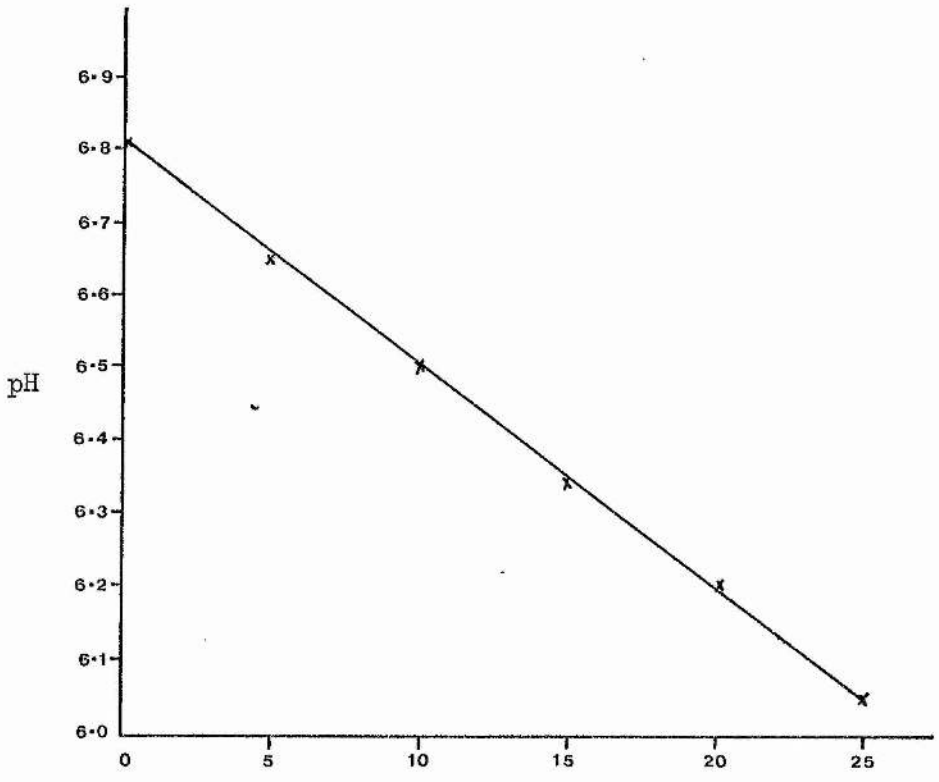


Fig. 3.31.3 A standard graph showing pH values for six levels of methionine



RESULTS

Table 3.31.1 Methionine content in mg. available methionine/g. dry bean for duplicate samples of seven varieties using three different inocula

Inoculum II

	Mexico 142	Sanilac	F.M52	Kabacuara	Pop412	W.C.1210	NEP 2
Sample a	0.87	2.29	1.00	2.49	1.77	1.00	1.93
Sample b	0.84	2.39	0.94	2.46	1.67	0.90	1.97
Mean	0.86	2.34	0.97	2.48	1.72	0.95	1.95

Analysis of Variance table for Inoculum II

Source	df	SS	MS	F	
Varieties	6	5.6400	0.9400	235.0**	
Samples	1	0.0021	0.0021	0.525	n.s.
Error	6	0.0245	0.0040		
Total	13	5.6666			

Inoculum III

	Mexico 142	Sanilac	EM52	Kabacuara	Pop412	W.C.1210	NEP 2
Sample a	0.69	1.84	0.66	1.93	1.48	0.60	1.51
Sample b	0.75	1.87	0.63	1.96	1.42	0.69	1.48
Mean	0.72	1.86	0.65	1.95	1.45	0.65	1.50

Table 3.31.1 (contd.)

Analysis of Variance table for Inoculum III

Source	df	SS	MS	F	
Varieties	6	3.9240	0.6540	436.0**	
Samples	1	0.0005	0.0005	0.3333	n.s.
Error	6	0.0090	0.0015		
Total	13	3.9335			

Inoculum IV

	Mexico 142	Sanilac	EM52	Kabacuara	Pop412	W.C.1210	NEP 2
Sample a	0.70	1.81	0.70	1.91	1.51	0.63	0.43
Sample b	0.72	1.91	0.75	2.00	1.45	0.66	0.50
Mean	0.71	1.86	0.73	1.96	1.48	0.65	0.47

Analysis of Variance table for Inoculum IV

Source	df	SS	MS	F	
Varieties	6	4.7064	0.7844	522.9**	
Samples	1	0.0064	0.0064	4.266	n.s.
Error	6	0.0088	0.0015		
Total	13	4.7216			

**indicates significance at the 1% level

n.s. indicates non significance

Table 3.31.2 Methionine content in mg. available methionine/g. dry bean - A comparison between different batches of inocula (each value is an average of duplicate samples)

Inoculum	Mexico 142	Sanilac	F.M.52	Kabacuara	Pop412	W.C.1210	NEP 2
II	0.86	2.34	0.97	2.48	1.72	0.92	1.95
III	0.72	0.65	0.65	1.95	1.45	0.65	1.50
IV	0.71	1.86	0.73	1.96	1.48	0.65	1.47

Analysis of Variance

Source	df	SS	MS	F
Varieties	6	6.3633	1.0605	16.5886**
Inocula	2	0.8928	0.4465	6.9532**
Error	12	0.7711	0.0642	
Total	20	8.0272		

** indicates significance at the 1% level

Table 3.31.3 To show the similarity in ranking order of the methionine results mg. available methionine/g. dry bean for the three inocula

	Mexico 142	Sanilac	EM52	Kabacuara	Pop412	WC1210	NEP2
Inoculum II	0.86	2.34	0.97	2.48	1.72	0.95	1.95
Rank	7	2	5	1	4	5	3
Inoculum III	0.72	1.86	0.65	1.95	1.45	0.65	1.50
Rank	5	2	6=	1	4	6=	3
Inoculum IV	0.71	1.86	0.73	1.96	1.48	0.65	0.47
Rank	5	2	4	1	3	6	7

Discussion

The results show that there are no significant differences between duplicate samples for a single inoculum. Two more inocula batches were tested to see if there were any significant differences in methionine content of the same varieties when tested with different inocula. Table 3.31.2 shows the means of duplicate samples tested with three different inocula, and also the analysis of variance.

The analysis of variance (Table 3.31.2) showed that (1) there were significant differences at the 1% level between the varieties and also that (2) there were significant differences at the 1% level in what was being measured as methionine content between the different inocula used.

Therefore, it could be concluded that the different inocula were not equally specific for methionine or there was some other nonspecific requirement of inoculum which was supplied by the beans. Thus indicating a difference in biological availability of the methionine in the beans. However, when the results of the different inocula were ranked, of the seven varieties, the low methionine varieties always remained low and the high methionine varieties high (see Table 3.31.3)

Thus, the Kelly method offers a rapid and relatively reliable test. The method is repeatable but not highly precise. It would therefore be a useful method for the evaluation of large populations in a breeding programme i.e. for a breeding programme it is only necessary to pick out the potentially significant differences in methionine levels among bean lines. The Kelly method would therefore be useful in this respect.

3.32 General Discussion and Conclusion

Both the microbiological techniques investigated were rapid and could be readily applied to large numbers of samples of dry beans. Both methods were also inexpensive - no expensive or sophisticated equipment was required. Either method could have therefore been used to detect any significant differences in the methionine level between the different varieties and could have been used to screen the large numbers of samples in a breeding programme.

However, difficulties arose with the Bolinder method because the amino acids for the medium were not of sufficient purity (see Appendix to section 3.3 of Chapter 3.). The Kelly method was therefore chosen since it gave reasonable consistency even if not certain quantitative accuracy. The results were therefore checked with the results on a E.E.C. amino acid analyser at Cambridge University. Since the amino acid analyser measures 'total' amino acid not "available", the results were higher but of corresponding range as shown in Table 3.31.4.

Table 3.31.4 Comparisons of methionine values (mg. methionine g. dry bean) using the Kelly microbiological technique and the automated amino acid analyser

Variety	Amino Acid Analyser	Rank	Kelly method	Rank
Mexico 142	2.90	5	0.77	5=
Sanilac	3.97	1	2.02	2
F.M.52	2.66	7	0.77	5=
Kabacuara	3.33	2	2.12	1
Pop 412	3.13	4	1.55	4
W.C.1210	3.08	5	0.72	7
NEP 2	3.26	3	1.64	3

CHAPTER 4 GENETIC VARIATION IN THE PROTEIN AND METHIONINE CONTENT OF THE VARIETIES

4.1 Introduction

In Uganda, the white haricot beans are not particularly well adapted to local environmental conditions, so the entire collection of white haricot beans were grown in the second rains of 1970, to examine the variability present. When once the limits of variability were defined, then selections could be made for varieties which had the desired combination of characters. These could then be used for the environmental and genetical studies.

4.2 Materials and methods

The entire collection of white haricot beans at Kabanyolo were grown out in the second rains of 1970. These consisted of a collection of white haricot lines from Tanzania (The Tengeru Collection); commercial varieties from Cambridge; lines selected from the World Collection and also lines from the breeding work at Kabanyolo.

In the field

The collection was planted at Kabanyolo farm on the 20th September, 1970. The land had been previously sown to maize and before planting was given a general dressing of 3 cwts. per acre of single superphosphate. The plots were unreplicated, each consisting of a double 10 ft. row, with an inter-row spacing of 2 ft. and an intra-row of 6 ins.

Each plant was recorded for germination, growth habit, rust, seed size, 100 seed weight and seed yield. Scoring for rust was carried out using a rapid visual assessment of the whole plot to which the disease rating of 0-5 was allocated. The different levels of rust reaction are listed below.

0 No sporing pustules

1 Rare pustules

2 Scattered pustules on a few leaves

3 Pustules fairly sparse, not on all leaves

4 Low density of pustules, distributed over most leaves, some plants visibly reduced in vigour.

5. Dense pustules over most leaves, often associated with severe chlorosis or isolated leaves with dense clusters of sporing pustules.

Details of the field characters are recorded in Appendix Table A4.3.1.

Analytical Techniques

(1) Crude Protein

Protein content was determined by the Kjeldahl technique as described in section 3.21 of Chapter 3. Sampling of the seed was carried out by bulking all the seed from each plot and taking a sample of 25 seeds. These were then dried, milled, and duplicate samples analysed in the usual way. The protein content was expressed as a % of the dry weight of the seed.

(2) Methionine

The methionine content was determined using the Kelly microbiological technique as described in section 3.31 of Chapter 3. The results are expressed as mg. methionine/g. dry bean.

4.3 Protein and methionine results

The protein and methionine results for the entire collection of white haricot beans are listed in Table 4.3.1. Protein is expressed as % crude protein, methionine as mg. available methionine per g. bean, both based on dry weight. An additional column of methionine as a percentage of the protein is included.

Table 4.3.1 Protein and methionine results for the entire collection of white haricot beans in September 1970

Kabanyolo No.	Tengeru No.	Variety	Origin	All values based on dry wt.		
				% crude protein	m.g. available methionine per g. bean	Methionine as a % of the protein
1	1	Antioquia 100	Bogota, Colombia	25.6	0.64	0.25
2	2	Cundin a Marca 12	" "	25.6	0.87	0.34
3	3	Cundin a Marca 64	" "	25.4	0.64	0.25
4	4	Bolivia 5	" "	26.3	0.64	0.24
5	7	Ecuador 16	" "	24.0	0.64	0.27
6	8	Ecuador 66	" "	25.9	0.87	0.34
7	9	Ecuador 68	" "	25.3	1.16	0.46
8	10	Ecuador 184	" "	21.9	0.87	0.40
9	13	Estados Unidos 113	" "	22.7	0.64	0.28
10	14	Mexico 119	" "	25.0	0.94	0.38
11	15	Mexico 137	" "	22.3	0.61	0.27
12	16	Mexico 142	" "	24.2	0.74	0.31
13	17	Mexico 162	" "	22.3	0.64	0.29
14	18	Mexico 184	" "	26.2	1.16	0.44
15	19	Mexico 200	" "	26.7	1.16	0.43
16	21	Mexico 483	" "	23.5	0.68	0.29
17	24	10064-4PM9PM-6PM	" "	22.6	0.64	0.28
18	25	Estados Unidos 112	" "	25.5	0.84	0.33
19	27	O.S.	T.F.A.	25.9	0.84	0.32
20	28	Idaho	H.Q.Dar es Salaam	23.3	0.74	0.32
21	29	Chilean	" " " "	25.8	0.68	0.26
22	30	Bombost	South Africa	27.7	1.13	0.40
23	31	Local (Ukiriguru)	Ukiriguru	21.0	0.61	0.29

Table 4.3.1 (contd.)

Kabanyolo No.	Tengeru No.	Variety	Origin	All values based on dry wt.		
				% crude protein	mg. available methionine per g. bean	Methionine as a % of the protein
24	32	Michigan	H.Q.Dar es Salaam	24.3	0.84	0.35
25	33	Unknown	Machingwea	24.3	0.64	0.26
26	35	Unknown	-	24.2	0.71	0.29
27	36	Sanilac	H.Q.Dar es Salaam	29.7	1.97	0.66
28	37	Ethiopian No.5	Ethiopia	23.4	0.61	0.26
29	39	U.S.Pea Bean No.3	U.S.	24.4	0.91	0.37
30	40	Ohtenashi	Japan	25.3	0.74	0.29
31	41	U.S.Pea Bean No.1	U.S.	25.9	0.97	0.37
32	42	Ethiopian No.2	Ethiopia	24.0	0.64	0.27
33	43	Chilean A	Chile	25.1	0.68	0.27
34	44	Chilean B	Chile	27.0	1.45	0.54
35	45	Chilean C	Chile	26.0	1.42	0.55
36	46	Ethiopian NA	Ethiopia	24.1	0.64	0.27
37	47	Ethiopia	Ethiopia	22.2	0.68	0.31
38	48	Ethiopia	Ethiopia	26.1	0.64	0.25
39	50	Guy	Belgium	25.2	0.74	0.28
40	51	Contessa	France	22.9	0.64	0.28
41	52	Ethiopia No.1	Ex Mitchell Cotts	26.9	1.16	0.43
42	53	Bonita	Puerto Rico	22.1	0.68	0.31
43	54	Criolla	Puerto Rico	21.3	0.71	0.33
44	55	Michigan	South Africa	24.4	0.87	0.36
45	57	Sanilac	Michigan State Univ.	29.3	1.87	0.64
46	58	Seaway	" " "	26.0	1.74	0.67
47	59	Michelite	" " "	26.3	1.55	0.59
48	60	Rice Pearl	Arusha	23.0	0.87	0.38

Table 4.3.1 (contd.)

Kabanyolo No.	Tengeru No.	Variety	Origin	All values based on dry wt. % crude protein	mg. available methionine per g. bean	Methionine as a % of protein
49	67	GN U.S. 1140	Univ. of Idaho	24.4	0.84	0.34
50	68	Small White UI 74	" " "	22.3	0.68	0.30
51	69	Calif. Small White	Brisbane, Queensland	22.8	0.64	0.28
52	70	Calif. Small White	" "	20.9	0.61	0.29
53	72	Small White FM. 51	Ferry Morse Seed Co.	21.6	0.64	0.30
54	73	" " FM. 52	" " "	20.3	0.64	0.32
55	74	" " FM. 53	" " "	20.2	0.64	0.32
56	75		Local collection, Tanzania	26.0	1.16	0.45
57	76		" "	22.6	0.68	0.30
58	77		" "	24.6	0.64	0.26
59	78		" "	24.6	0.64	0.26
60	79		" "	21.2	0.64	0.30
61	80		" "	24.5	0.71	0.29
62	81		" "	23.1	0.64	0.28
63	82		" "	27.1	0.97	0.36
64	83		" "	25.0	0.78	0.31
65	84		" "	25.3	0.68	0.27
66	85		" "	24.1	0.64	0.27
67	86		" "	23.3	0.64	0.27
68	87		" "	24.1	0.64	0.27
69	88		" "	25.2	0.64	0.25
70	89		" "	20.4	0.61	0.30
71	90		" "	27.4	1.87	0.68
72	92		" "	——	lost	——

Table 4.3.1 (contd.)

Kabanyolo No.	Tengeru No.	Variety	Origin	All values based on dry wt. % crude protein	mg. available methionine per g. bean	Methionine as a % of protein
73	93		Local Collection Tanzania	25.0	0.84	0.34
74	94		" "	23.4	0.64	0.27
75	95		" "	23.4	0.64	0.27
76	96		" "	23.1	0.64	0.28
77	97		" "	23.4	0.64	0.27
78	98		" "	24.1	0.68	0.28
79	99		" "	24.7	0.61	0.25
80	100		" "	26.1	0.68	0.26
81		Sanilac	Cambridge	29.1	1.87	0.64
82		Seaway	"	25.2	1.78	0.71
83		Michelite	"	26.4	1.74	0.66
84		0154	World Collection	21.5	0.61	0.28
85		1509	Cambridge (ex USDA/USAID)	23.2	0.74	0.32
86		1210	" "	23.4	0.68	0.29
87		1488	" "	22.5	0.61	0.27
88		1058	" "	22.3	0.61	0.27
89		1046	" "	21.5	0.94	0.44
90		1073	" ""	25.3	0.87	0.34
91		Predome nain	France, (Bannerot)	25.7	0.94	0.37
92		Pronel	" "	24.7	1.52	0.62
93		Ocop 9X	" "	21.3	1.45	0.68

Table 4.3.1 (contd.)

Kabanyolo No.	Tengeru No.	Variety	Origin	All values based on dry wt. % crude protein	mg. available methionine per g. bean	Methionine as a % of protein
94		Pop 412	Prelude (Processor x Cornell 49-242)	28.3	1.45	0.51
95		Verdon	France, (Bannerot)	25.2	1.45	0.58
96		NEP 2	Mutation from San Fernando	27.6	1.55	0.56
97		Cuarentino	INEAC Rwanda	24.2	1.49	0.62
98		Kabacuara	Kabanyolo selection by Leakey	26.4	1.94	0.73
99		Emerson 847	Michigan State Univ.	25.7	1.16	0.45
100		Perry Marrow	" " "	25.6	1.20	0.47
101		S1	White seeded selections from Leakey's breeding work at Kabanyolo	22.7	0.61	0.27
102		S2		23.8	0.64	0.28
103		S3		24.1	0.64	0.27
104		S4	" "	25.2	0.74	0.29
105		S5	" "	21.8	0.64	0.29
106		S6	" "	23.1	0.61	0.26
107		S7	" "	23.9	0.64	0.27
108		S8	" "	-	Lost	-
109		S9	" "	24.7	0.64	0.26
110		S10	" "	23.7	0.68	0.29
111		S11	" "	22.4	0.64	0.29
112		S12	" "	22.7	0.64	0.28
113		S13	" "	22.5	0.64	0.28
114		S14	" "	24.0	0.68	0.28
115		S15	" "	21.8	0.61	0.28
116		S16	" "	21.0	0.61	0.29
117		S17	" "	20.6	0.64	0.31

Table 4.3.1 (contd.)

Kabanyolo No.	Tengeru No.	Variety	Origin	All values based on dry wt. % crude protein	mg. available methionine per g. bean	Methionine as a % of protein
118		S18	White seeded selections from Leakey's breeding work at Kabanyolo	20.7	0.64	0.31
119		S19		——	Lost	——
120		S20		22.9	0.68	0.30
121		S21	" "	20.8	0.64	0.31
122		S22	" "	20.3	0.64	0.31
123		S23	" "	23.4	0.64	0.27
124		S24	" "	22.9	0.64	0.28
125		S25	" "	24.2	0.68	0.28
126		S26	" "	24.3	0.68	0.28
127		S27	" "	24.4	0.68	0.28
128		S28	" "	23.0	0.68	0.30
129		S29	" "	25.4	0.68	0.27
130		S30	" "	26.0	0.68	0.26
131		S31	" "	24.8	0.64	0.26
132		S32	" "	24.7	0.68	0.28
133		S33	" "	23.3	0.68	0.29
134		S34	" "	22.0	0.61	0.28
135		S35	" "	24.1	0.64	0.27
136		S36	" "	24.2	0.64	0.26
137		S37	" "	26.9	0.68	0.25
138		S38	" "	25.4	0.68	0.27
139		S39	" "	23.2	0.64	0.28
140		S40	" "	22.1	0.61	0.28
141		S41	" "	24.0	0.68	0.28
142		S42	" "	23.8	0.68	0.29
143		S43	" "	23.6	0.68	0.29

Table 4.3.1 (contd.)

Kabanyolo No.	Tengeru No.	Variety	Origin	All values based on dry wt. % crude protein	mg. available methionine per g. bean	Methionine as a % of protein
144		S44	White seeded selections from Leakey's breeding work at Kabanyolo	21.4	0.64	0.30
145		S45	" "	23.5	0.64	0.27
146		S46	" "	21.8	0.64	0.29
147		S47	" "	21.1	0.64	0.30
148		S48	" "	22.0	0.64	0.29
149		S49	" "	23.8	0.68	0.29
150		S50	" "	24.6	0.68	0.28
151		S51	" "	25.0	0.68	0.27
152		S52	" "	24.7	0.64	0.26
153		S53	" "	24.9	0.64	0.26
154		S54	" "	26.8	1.13	0.42
155		S55	" "	26.9	1.20	0.44
156		S56	" "	25.3	1.13	0.45
157		S57	" "	27.9	1.65	0.59
158		S58	" "	25.7	1.20	0.47
159		S59	" "	25.0	1.20	0.48
160		S60	" "	25.8	1.16	0.45
161		S61	" "	24.1	0.68	0.28
162		S62	" "	24.5	0.68	0.28
163		S63	" "	25.6	0.68	0.27
164		S64	" "	25.9	0.68	0.26
165		S65	" "	24.8	0.68	0.27
166		S66	" "	26.9	1.58	0.59

Table 4.3.1 (contd.)

Kabanyolo No.	Tengeru No.	Variety	Origin	All values based on dry wt. % crude protein	mg. available methionine per g. bean	Methionine as a % of protein
167		S67	White seeded selections from Leakey's breeding work at Kabanyolo	26.0	1.20	0.46
168		S68	" "	25.1	1.16	0.46
169		S69	" "	-	Lost	-
170		S70	" "	26.0	1.20	0.46
171		S71	" "	25.6	1.16	0.45
172		S72	" "	-	Lost	-
173		S73	" "	-	Lost	-

Table 4.3.2 Characters of the twenty five varieties selected from the entire collection of white haricot beans at Kabanyolo

Variety	Habit	Rust	Seed Size mm.	100 Seed Wt. g.	Seed Yield g./ plant	% Crude Protein	mg. Methionine /g. bean
Cundin a Marca	IV	O	S	H	L	M	L
Ecuador 66	IV	O	M	M	M	M	L
Ecuador 68	IV	O	S	L	M	M	M
Ecuador 184	IV	R	M	L	M	L	L
Mexico 119	IV	O	M	M	H	M	L
Mexico 187	IV	O	S	M	M	L	L
Mexico 142	IV	R	S	L	M	M	L
Mexico 184	IV	O	M	M	M	M	M
Mexico 200	IV	O	S	L	M	M	M
Sanilac	D	R	S	L	L	H	H
Seaway	D	R	S	L	L	M	H
EM52	IV	R	S	L	M	L	L
EM53	IV	R	S	L	M	L	L
Var.77	IV	R	S	L	M	M	L
Kabacuara	IB	O	S	L	M	M	H
Cuarentino	IB	R	S	L	H	M	M
NEP 2	IB	O	S	L	H	H	H
Pronel	D	R	L	M	M	M	H
Ocop 9X	D	R	L	M	L	L	M
Pop 412	D	R	M	L	M	H	M
Verdon	D	R	M	M	L	M	M
WC. 1210	IV	R	S	L	M	M	L
WC. 1509	D	R	S	L	M	M	L
WC. 1046	D	R	M	L	M	L	L
Var. 100	IV	R	M	H	L	M	M

4.4 Discussion and Conclusion

The percentage crude protein content of the different varieties of the entire collection varied between 20.2 and 29.7%. The methionine content varied between 0.61 and 1.97 mg. available methionine/g. dry bean. The column - methionine as a % of the protein - in Table 4.3.1. indicates that in general high protein varieties have a high methionine content and low protein varieties in general have a low methionine content. Thus when selecting for high protein content one may also be selecting for high methionine.

From this collection of white haricot beans, twenty five varieties were selected on the basis of their having some or all of the desirable characters required for canning. They are listed in Table 4.3.2.

For comparison, the ideal bean would have a determinate growth habit, (D); a zero rust reaction, (O); a small seed size, (S); a low 100 seed weight, (L); a high seed yield, (H); a high protein content, (H); and a high methionine content, (H).

It can be seen from the results in Table 4.3.2., that Sanilac, the commercially accepted variety, has all the desirable characters except that it is badly affected by rust and therefore also has a low yield when grown in Uganda. Similarly, Seaway is affected by rust and has a low yield and also only a medium protein content. Mexico 142, the commercially accepted variety in East Africa is also affected by rust and only has a medium yield and a medium protein content. The

most promising variety seems to be NEP2. It has all the desirable characters, though its seed size is not quite ideal. All the other varieties listed in Table 4.3.2. show widely differing characters. They were grown in a 5 x 5 lattice square in four subsequent seasons, to determine to what extent their characters varied or remained the same when grown in different seasons. Particular reference was made to protein and methionine content (see chapter 5).

Key to Table 4.3.2

D = determinate
I = indeterminate vine
IB = indeterminate bush

S = small
M = medium
L = large

L = low
H = high
O = rust resistance or tolerance
R = rust reaction

CHAPTER 5 ENVIRONMENTAL STUDIES ON THE PROTEIN AND METHIONINE CONTENT OF SELECTED BEAN VARIETIES

5.1 Introduction

Chapter 4 demonstrates that white haricot beans differ considerably in protein and methionine content, but to what extent the variation is genetic or environmental, it is not known. This chapter considers the effect of a change of season in field plots and also the effect of overall fertility level in pot tests, on the protein and methionine content.

Experiment 1 considers the differences in protein and methionine content of the same genotypes grown in four different seasons. Soil and climate may both contribute to any differences in performance.

Experiment 2 considers the effects of different mixed fertiliser regimes in pot tests on high, intermediate or low protein varieties.

5.2 Comparisons of the same genotypes in different seasons

5.21 Materials and methods

Twenty five varieties selected on the basis of their combination of desirable characters were grown in the years 1971 and 1972 i.e. in four different seasons. The varieties are listed in Fig. 5.21.1.

For each season, the same methods of preparation of the land were followed, and the land was treated with a general dressing of 3 cwts. per acre of single superphosphate.

Fig. 5.21.1 A list of the varieties grown in the four Variety Trials in 1971 - 1972

Variety Trial No.	Tengeru Collection No.	Variety
V1	T2	Cundin a Marca
V2	T8	Ecuador 66
V3	T9	Ecuador 68
V4	T10	Ecuador 184
V5	T14	Mexico 119
V6	T15	Mexico 137
V7	T16	Mexico 142
V8	T18	Mexico 184
V9	T19	Mexico 200
V10	T36	Sanilac
V11	T58	Seaway
V12	T73	EM52
V13	T74	EM53
V14	T77	Var.77
V15		Kabacuara
V16		Cuarentino
V17		NEP2
V18		Pronel
V19		Ocop 9X
V20		Pop 412
V21		Verdon
V22		W.C. 1210
V23		W.C. 1509
V24		W.C. 1046
V25		Var. 100

The Variety Trials were planted in the design of a 5 x 5 balanced lattice square with the replicates as shown in Fig. 5.21.2 (design taken from Cochran and Cox (1957) page 498).

Fig. 5.21.2 A 5 x 5 balanced lattice square for the Variety Trials

V = 25; K = 5; R = 3; Rows = 15; columns = 15

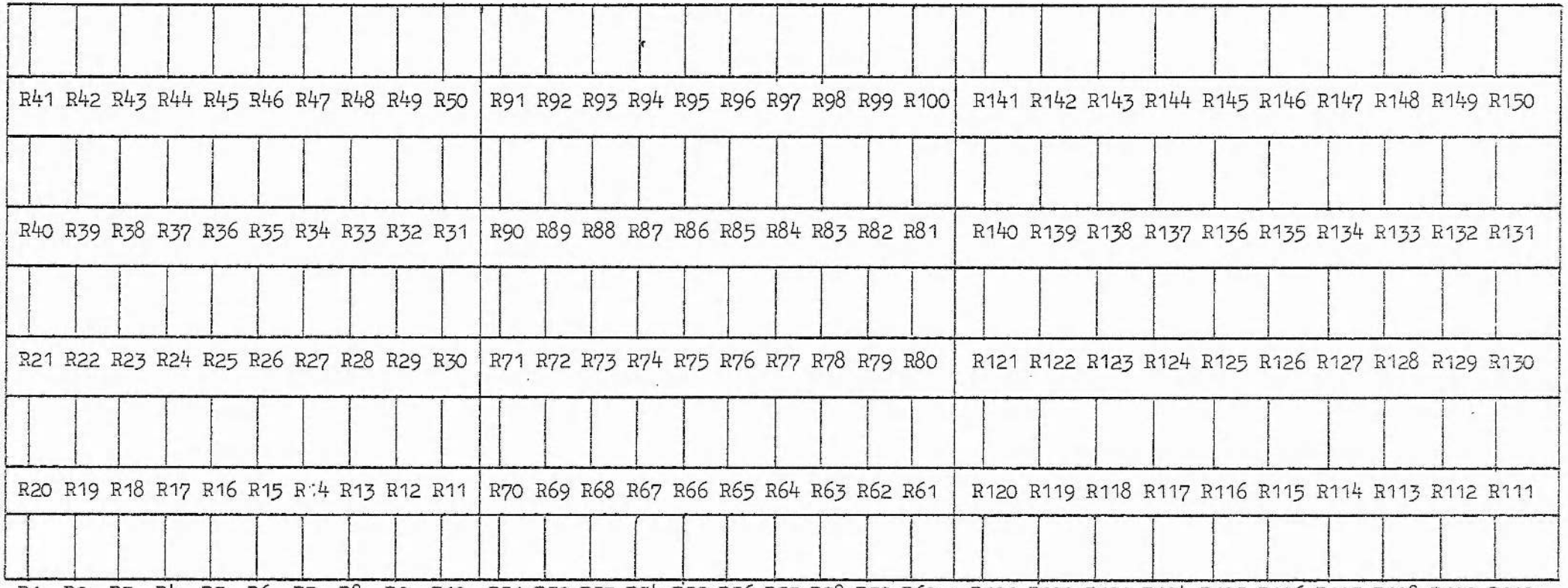
Rep 1	Rep 2	Rep 3
V1 V2 V3 V4 V5	V1 V10 V14 V18 V22	V1 V8 V15 V17 V24
V6 V7 V8 V9 V10	V23 V2 V6 V15 V19	V25 V2 V9 V11 V18
V11 V12 V13 V14 V15	V20 V24 V3 V7 V11	V19 V21 V3 V10 V12
V16 V17 V18 V19 V20	V12 V16 V25 V4 V8	V13 V20 V22 V4 V6
V21 V22 V23 V24 V25	V9 V13 V17 V21 V5	V7 V14 V16 V23 V5

Key

V = varieties
K = square value
R = replicates

The field plan for each of the Variety Trials is shown in Fig. 5.21.3

Fig. 5.21.3 A field plan for the Variety Trials grown in the four different seasons



Key G = Guard row
R = row

In the field

Each variety was grown in a single plot in each replicate. A plot consisted of a double 10 ft. row, with an inter-row spacing of 2 ft., and an intra-row of 6 ins. Each plot was scored for the usual characters - growth habit, rust reaction, total seed yield, average seed size, and 100 seed weight. These results are recorded in Appendix Tables A5.21.1 - A5.21.4.

Analytical Techniques

(1) Protein content

The percentage crude protein content was determined using the Kjeldahl technique as described in section 3.21 of chapter 3. Sampling of the seed was carried out by bulking all the seed from each plot within a replicate and taking a sample of 25 seeds. These were then dried, milled and duplicate samples analysed in the usual way. The protein content was expressed as a percentage of the dry weight of the seed. The results are shown in Tables 5.22.1 - 5.22.4.

(2) Methionine

The methionine content was determined using the Kelly microbiological technique as described in section 3.31 of chapter 3, using the same dried milled sample that was used for the protein analysis. The sample was analysed in duplicate and expressed as mg. methionine/g. bean.

5.22 Protein results

Table 5.22.1 Percentage crude protein results for the twenty five varieties grown in the 1st Rains of 1971

Variety No.	Variety	Rep. 1	Rep. 2	Rep. 3	Means
V1	Cundin a Marca	22.4	21.9	24.6	23.0
V2	Ecuador 66	24.3	24.3	24.0	24.2
V3	Ecuador 68	26.5	26.9	27.8	27.1
V4	Ecuador 184	23.9	25.6	24.4	24.6
V5	Mexico 119	25.9	23.2	23.8	24.3
V6	Mexico 137	22.7	23.7	22.0	22.8
V7	Mexico 142	24.2	25.7	21.7	25.0
V8	Mexico 184	24.9	25.0	27.5	25.8
V9	Mexico 200	26.1	22.5	23.9	24.2
V10	Sanilac	25.2	24.6	24.6	24.8
V11	Seaway	21.9	23.7	22.5	22.7
V12	EM52	19.6	19.3	21.2	20.0
V13	EM53	20.8	21.6	20.7	21.0
V14	Var.77	24.6	25.6	25.0	25.1
V15	Kabacuara	28.3	29.9	28.3	28.8
V16	Cuarentino	20.5	23.2	23.7	22.5
V17	NEP 2	25.2	25.7	27.8	26.2
V18	Pronel	22.7	23.6	25.9	24.1
V19	Ocop 9X	20.6	21.4	22.0	21.3
V20	Pop 412	26.2	23.9	24.3	24.8
V21	Verdon	24.6	25.8	24.2	24.9
V22	W.C. 1210	24.2	24.3	25.4	24.6
V23	W.C. 1509	22.9	22.3	23.9	23.0
V24	W.C. 1046	24.1	24.6	25.1	24.6
V25	Var. 100	25.8	24.2	23.8	24.6

Table 5.22.1 contd.

Analysis of Variance Table

Source	df	SS	MS	F	
Replications	2	2.0096	1.0048	0.6058	n.s.
Varieties	24	253.9741	10.5822	6.3805**	
Row (adj.)	12	13.0249	1.0854 Er		
Col.(adj.)	12	8.5469	0.7122 Ec		
Error	24	39.8053	1.6585 Ee		
Total	74	317.3608			

** indicates significance at the 1% level.

Er and Ec are less than Ee, therefore there is no need for adjustment of row and column effects.

n.s. indicates non significance

Table 5.22.2 Percentage crude protein results for the twenty five varieties grown in the 2nd Rains of 1971

Variety No.	Variety	Rep 1	Rep 2	Rep 3	Means
V1	Cundin a Marca	22.5	23.5	24.2	23.4
V2	Ecuador 66	24.0	24.3	24.4	24.2
V3	Ecuador 68	26.4	25.6	25.5	25.8
V4	Ecuador 184	26.6	26.1	25.3	26.0
V5	Mexico 119	24.7	23.8	24.5	24.3
V6	Mexico 137	23.4	25.4	23.8	24.2
V7	Mexico 142	24.3	25.0	24.8	24.7
V8	Mexico 184	27.8	27.2	26.6	27.2
V9	Mexico 200	27.8	25.4	27.8	27.0
V10	Sanilac	28.7	29.1	28.0	28.6
V11	Seaway	27.1	26.4	26.3	26.6
V12	EM.52	21.9	21.3	21.7	21.6
V13	EM.53	22.1	22.8	21.1	22.0
V14	Var. 77	26.3	24.9	25.9	25.7
V15	Kabacuara	27.8	28.5	27.6	28.0
V16	Cuarentino	25.5	25.6	27.1	26.1
V17	NEP 2	28.7	26.3	27.8	27.6
V18	Pronel	28.5	26.7	26.4	27.2
V19	Ocop 9X	25.4	23.4	23.5	24.1
V20	Pop 412	25.4	25.6	25.3	25.4
V21	Verdon	26.8	28.4	27.3	27.5
V22	W.C.1210	24.4	24.8	25.5	24.9
V23	W.C.1509	26.6	24.7	26.2	25.8
V24	W.C.1046	25.8	26.8	25.3	26.0
V25	Var. 100	26.5	24.5	24.2	25.1

Table 5.22.2 (contd.)

Analysis of Variance Table

Source	df	SS	MS	F	
Replications	2	2.1168	1.0584	1.4279	n.s.
Varieties	24	221.1533	9.2147	12.4321**	
Row (adj.)	12	5.8036	0.4836Er		
Col. (adj.)	12	6.7956	0.5663Ec		
Error	24	17.7907	0.7412Ee		
Total	74	253.66			

** indicates significance at the 1% level. Er and Ec are less than Ee, therefore there is no need for adjustment of row and column effects.

n.s. indicates non significance

Table 5.22.3 Percentage crude protein results for the twenty five varieties grown in the 1st Rains of 1972

Variety No.	Variety	Rep 1	Rep 2	Rep 3	Means
V1	Cundin a Marca	24.5	23.7	23.5	23.9
V2	Ecuador 66	25.5	27.8	27.7	27.0
V3	Ecuador 68	25.3	26.1	24.5	25.3
V4	Ecuador 184	25.5	23.5	24.9	24.6
V5	Mexico 119	25.1	25.9	24.3	25.1
V6	Mexico 137	25.2	24.5	23.2	24.3
V7	Mexico 142	23.6	25.9	26.2	25.2
V8	Mexico 184	25.4	24.9	24.6	25.0
V9	Mexico 200	25.6	23.9	25.9	25.1
V10	Sanilac	25.9	26.3	24.4	25.5
V11	Seaway	23.4	23.9	24.1	23.8
V12	EM. 52	22.3	21.1	21.1	21.5
V13	EM. 53	22.9	23.1	23.7	23.2
V14	Var. 77	27.3	26.5	25.3	26.4
V15	Kabacuara	28.2	26.8	28.5	27.8
V16	Cuarentino	23.5	23.3	24.6	23.8
V17	NEP 2	26.3	27.1	26.5	26.6
V18	Pronel	24.8	24.9	25.0	24.9
V19	Ocop 9x	21.0	21.7	21.0	21.2
V20	Pop 412	24.1	26.1	24.6	24.9
V21	Verdon	23.7	24.5	23.5	23.9
V22	W.C. 1210	25.5	27.8	27.2	26.8
V23	W.C. 1509	26.8	25.4	24.7	25.6
V24	W.C. 1046	26.5	24.0	26.4	25.6
V25	Var. 100	25.1	24.8	24.2	24.7

Table 5.22.3 (Contd.)

Analysis of Variance Table

Source	df	SS	MS	F	
Replications	2	0.3603	0.1801	0.2435	n.s.
Varieties	24	169.2605	7.0525	9.5355**	
Row (adj.)	12	4.8516	0.4043Er		
Col.(adj.)	12	15.6102	1.3008Ec		
Error	24	17.7313	0.7396Ee		
Total	74	207.8339			

**indicates significance at the 1% level,
Er is less than Ee, therefore there is
no need for adjustment of row effects.
Ec is greater than Ee therefore adjustments
of column effects have been made.

n.s. indicates non significance

Table 5.22.4 Percentage crude protein results for the twenty five varieties grown in the 2nd Rains of 1972

Variety No.	Variety	Rep 1	Rep 2	Rep 3	Means
V1	Cundin a Marca	24.0	23.1	23.6	23.6
V2	Ecuador 66	28.4	28.5	26.7	27.9
V3	Ecuador 68	26.8	24.2	27.6	26.2
V4	Ecuador 184	25.2	25.8	24.9	25.3
V5	Mexico 119	24.8	25.8	24.4	25.0
V6	Mexico 137	24.8	24.0	22.0	23.6
V7	Mexico 142	24.2	25.5	25.3	25.0
V8	Mexico 184	26.5	27.8	27.3	27.2
V9	Mexico 200	27.7	27.3	27.3	27.4
V10	Sanilac	29.3	30.5	29.3	30.0
V11	Seaway	26.9	26.8	26.9	26.9
V12	EM.52	21.6	20.9	21.9	21.5
V13	EM.53	23.3	23.1	23.0	23.1
V14	Var. 77	25.3	25.9	24.9	25.4
V15	Kabacuara	28.7	28.2	28.9	28.6
V16	Cuarentino	26.1	25.9	26.1	26.0
V17	NEP 2	27.7	26.9	26.7	27.1
V18	Pronel	29.4	29.2	28.5	29.0
V19	Ocop 9X	26.9	27.8	27.9	27.5
V20	Pop 412	27.6	27.8	27.9	27.8
V21	Verdon	28.0	27.8	27.6	27.8
V22	W.C.1210	25.6	24.9	24.2	24.9
V23	W.C.1509	25.8	25.3	26.0	25.7
V24	W.C.1046	25.3	25.8	26.8	26.0
V25	Var. 100	25.6	25.3	25.8	25.6

Table 5.22.4 (Contd.)

Analysis of Variance Table

Source	df	SS	MS	F	
Replications	2	0.3300	0.1650	0.3497	n.s.
Varieties	24	274.8800	11.4533	24.2757**	
Row (adj.)	12	8.1309	0.6775Er		
Col.(adj.)	12	3.1649	0.2637Ec		
Error	24	11.3242	0.4718Ee		
Total	74	297.8300			

**indicates significance at the 1% level.

Ec is less than Ee therefore no adjustment for column effects. Er is greater than Ee therefore adjustments of row effects have been made.

n.s. indicates non significance

Table 5.22.5 Percentage crude protein results for the comparison of four seasons Variety Trials (each value is an average of 3 replicates)

Variety No.	Variety	1st Rains 1971	2nd Rains 1971	1st Rains 1972	2nd Rains 1972
V1	Cundin a Marca	22.97	23.40	23.90	23.56
V2	Ecuador 66	24.20	24.23	27.00	27.86
V3	Ecuador 68	27.07	25.83	25.30	26.20
V4	Ecuador 184	24.63	26.00	24.63	25.30
V5	Mexico 119	24.30	24.33	25.10	25.00
V6	Mexico 137	22.80	24.20	24.30	23.60
V7	Mexico 142	24.95	24.70	25.23	25.00
V8	Mexico 184	25.80	27.20	24.97	27.20
V9	Mexico 200	24.17	27.00	25.13	27.43
V10	Sanilac	24.80	28.60	25.53	29.70
V11	Seaway	22.70	26.60	23.80	26.86
V12	EM. 52	20.03	21.63	21.50	21.46
V13	EM. 53	21.03	22.00	23.23	23.13
V14	Var.77	25.07	25.70	26.37	25.36
V15	Kabacuara	28.83	27.97	27.83	28.60
V16	Cuarentino	22.47	26.07	23.80	26.03
V17	NEP 2	26.23	27.60	26.63	27.10
V18	Pronel	24.07	27.20	24.90	29.03
V19	Ocop 9X	21.33	24.10	21.23	27.53
V20	Pop 412	24.80	25.43	24.93	27.76
V21	Verdon	24.87	27.50	23.90	27.80
V22	W.C.1210	24.63	24.90	26.83	24.90
V23	W.C. 1509	23.03	25.83	25.63	25.70
V24	W.C.1046	24.60	25.97	25.63	25.96
V25	Var. 100	24.60	25.07	24.70	25.56

Table 5.22.5 (contd.)

Analysis of Variance Table

Source	df	SS	MS	F
Varieties	24	671.93	27.99	45.66**
Seasons	3	185.17	61.72	100.69**
Varieties x Seasons	72	256.70	3.565	5.82**
Error	200	122.59	0.613	
Total	299	1236.39		

** indicates significance at 1% level

5.23 Discussion of protein results

The analyses of variances for the 5 x 5 lattice square designs (Tables 5.22.1 - 5.22.4) for each of the four seasons has shown that in all cases:

(1) there were no significant differences in protein content between plants of the same variety grown in different replicates.

(2) there were significant differences in protein content between the different varieties at the 1% level.

The results were then analysed to see if there were any seasonal effects on protein content. Table 5.22.5 shows the mean protein results and the Analysis of Variance table. The analysis shows that there were significant differences in percentage crude protein content between varieties, seasons and varieties x seasons interaction. The significant variety x season interaction indicates that there are significant environmental effects on protein content.

5.24 Methionine results

Table 5.24.1 Methionine results (mg. available methionine / g.dry bean) for the twenty five varieties grown in the 1st Rains of 1971 (each value is a mean of duplicate analytical samples)

Variety	Variety Trial No.	Rep 1	Rep 2	Rep 3
Cundin a Marca	V1	0.67	0.63	0.67
Ecuador 66	V2	0.86	0.84	0.84
Ecuador 68	V3	0.92	0.95	0.97
Ecuador 184	V4	0.86	0.87	0.86
Mexico 119	V5	0.84	0.84	0.86
Mexico 137	V6	0.65	0.66	0.65
Mexico 142	V7	0.73	0.63	0.65
Mexico 184	V8	1.18	1.13	1.15
Mexico 200	V9	1.18	1.17	1.18
Sanilac	V10	1.87	1.85	1.87
Seaway	V11	1.44	1.46	1.41
F.M.52	V12	0.68	0.65	0.65
F.M.53	V 13	0.65	0.66	0.58
Var.77	V14	1.47	1.46	1.42
Kabacuara	V15	1.93	1.96	1.95
Cuarentino	V16	1.51	1.49	1.44
NEP 2	V17	1.52	1.58	1.51
Pronel	V18	1.52	1.55	1.44
Ocop 9X	V19	0.65	0.70	0.65
Pop 412	V20	1.46	1.46	1.51
Verdon	V21	1.44	1.46	1.47
W.C. 1210	V22	0.68	0.68	0.74
W.C. 1509	V23	0.73	0.80	0.74
W.C. 1046	V24	0.99	0.95	0.96
Var. 100	V25	0.74	0.71	0.81

Table 5.24.1 (Contd.)

Analysis of Variance Table

Source	df	SS	MS	F	
Varieties	24	12.335	0.514	514.0**	
Replicates	2	0.001	0.001	1.00	n.s.
Error	48	0.055	0.001		
Total	74	12.391			

** indicates significance at the 1% level

n.s. indicates non signfficance

Table 5.24.2 Methionine results (mg. available methionine/g. dry bean) for the twenty five varieties grown in the 2nd Rains of 1971 (each value is a mean of duplicate analytical samples)

Variety	Variety Trial No.	Rep 1	Rep 2	Rep 3
Cundin a Marca	V1	0.75	0.77	0.77
Ecuador 66	V2	0.89	0.93	0.93
Ecuador 68	V3	1.02	0.97	1.00
Ecuador 184	V4	0.34	0.24	0.29
Mexico 119	V5	0.91	0.96	0.91
Mexico 137	V6	0.67	0.65	0.56
Mexico 142	V7	0.45	0.48	0.45
Mexico 184	V8	1.34	1.37	1.37
Mexico 200	V9	1.19	1.18	1.14
Sanilac	V10	1.92	1.97	1.94
Seaway	V11	0.55	0.57	0.52
F.M.52	V12	0.86	0.84	0.89
F.M.53	V13	0.67	0.84	0.65
Var.77	V14	1.22	1.43	1.50
Kabacuara	V15	1.94	1.98	1.95
Cuarentino	V16	1.77	1.77	1.50
NEP 2	V17	1.56	1.59	1.54
Pronel	V18	1.15	1.18	1.22
Ocop 9X	V19	1.22	1.24	1.27
Pop 412	V20	1.54	1.62	1.62
Verdon	V21	1.34	1.35	1.37
W.C.1210	V22	0.60	0.66	0.62
W.C.1509	V23	0.52	0.62	0.61
W.C.1046	V24	0.99	1.00	0.91
Var.100	V25	0.75	0.80	0.72

Table 5.24.2 (Contd.)

Analysis of Variance Table

Source	df	SS	MS	F	
Varieties	24	15.185	0.632	210.7**	
Replicates	2	0.181	0.009	3.00	n.s.
Error	48	0.150	0.003		
Total	74	15.326			

**indicates significance at the 1% level

n.s. indicates non significance

Table 5.24.3 Methionine results (mg. available methionine/g.dry bean) for the twenty five varieties grown in the 1st Rains of 1972 (each value is an average of duplicate analytical samples)

Variety	Variety Trial No.	Rep 1	Rep 2	Rep 3
Cundin a Marca	V1	0.70	0.75	0.72
Ecuador 66	V2	0.88	0.86	0.85
Ecuador 68	V3	1.02	1.99	1.40
Ecuador 184	V4	0.84	0.81	0.88
Mexico 119	V5	0.85	0.85	0.87
Mexico 137	V6	0.47	0.46	0.47
Mexico 142	V7	0.65	0.65	0.60
Mexico 184	V8	1.21	1.19	1.19
Mexico 200	V9	1.21	1.17	1.21
Sanilac	V10	1.98	1.90	1.86
Seaway	V11	1.42	1.43	1.44
F.M.52	V12	0.43	0.48	0.45
F.M.53	V13	0.47	0.65	0.63
Var. 77	V14	1.55	1.37	1.45
Kabacuara	V15	1.64	1.98	1.95
Cuarentino	V16	1.47	1.45	1.50
NEP 2	V17	1.33	1.34	1.39
Pronel	V18	1.54	1.55	1.56
Ocop 9X	V19	0.84	0.88	0.91
Pop 412	V20	1.24	1.30	1.18
Verdon	V21	1.62	1.75	1.64
W.C. 1210	V22	0.64	0.72	0.65
W.C. 1509	V23	0.94	0.64	0.90
W.C. 1046	V24	0.96	0.99	0.99
Var. 100	V25	0.75	0.70	0.72

Table 5.24.3 (Contd.)

Analysis of Variance Table

Source	df	SS	MS	F	
Varieties	24	13.404	0.559	39.93**	
Replicates	2	0.030	0.015	1.071	n.s.
Error	48	0.661	0.014		
Total	74				

** indicates significance at the 1% level

n.s. indicates non significance

Table 5.24.4 Methionine results (mg. available methionine/g.dry bean) for the twenty five varieties grown in the 2nd Rains of 1972 (each value is an average of duplicate analytical samples)

Variety	Variety Trial No.	Rep 1	Rep 2	Rep 3
Cundin a Marca	V1	0.85	0.87	0.87
Eucador 66	V2	1.00	1.10	0.98
Ecuador 68	V3	1.42	1.41	1.47
Ecuador 184	V4	1.38	1.31	1.38
Mexico 119	V5	1.27	1.25	1.24
Mexico 137	V6	0.82	0.82	0.85
Mexico 142	V7	0.75	0.82	0.85
Mexico 184	V8	1.55	1.54	1.60
Mexico 200	V9	1.58	1.53	1.57
Sanilac	V10	2.36	2.33	2.33
Seaway	V11	1.79	1.74	1.77
F.M.52	V12	1.18	1.18	1.15
F.M.53	V13	0.93	0.95	0.92
Var.77	V14	1.64	1.63	1.44
Kabacuara	V15	2.43	2.44	2.25
Cuarentino	V16	1.70	1.68	1.67
NEP 2	V17	1.73	1.71	1.73
Pronel	V18	1.64	1.64	1.70
Ocop 9X	V19	1.48	1.46	1.50
Pop 412	V20	1.81	1.86	1.89
Verdon	V21	1.84	1.88	1.86
W.C. 1210	V22	0.70	0.72	0.72
W.C. 1509	V23	0.93	0.97	0.93
W.C. 1046	V24	1.00	1.10	1.07
Var. 100	V25	0.64	0.72	0.79

Table 5.24.4 (Contd.)

Analysis of Variance Table

Source	df	SS	MS	F
Varieties	24	16.143	0.673	336.5**
Replicates	2	0	0	
Error	48	0.106	0.002	
Total	74	16.249		

** indicates significance at the 1% level

Table 5.24.5 Comparison of the methionine content (mg. available methionine/g. dry bean) for the four Variety Trials (each value is an average of three replicates)

Variety	Variety Trial No.	1st rains 1971	2nd rains 1971	1st rains 1972	2nd rains 1972
Cundin a Marca	V1	0.65	0.76	0.72	0.86
Ecuador 66	V2	0.85	0.91	0.86	1.03
Ecuador 68	V3	0.95	0.97	1.13	1.44
Ecuador 184	V4	0.86	0.29	0.84	1.35
Mexico 119	V5	0.85	0.92	0.86	1.25
Mexico 137	V6	0.65	0.63	0.46	0.83
Mexico 142	V7	0.67	0.46	0.63	0.81
Mexico 184	V8	1.15	1.36	1.20	1.56
Mexico 200	V9	1.18	1.17	1.19	1.56
Sanilac	V10	1.86	1.94	1.91	2.34
Seaway	V11	1.43	0.55	1.43	1.76
F.M.52	V12	0.66	0.86	0.45	1.16
F.M.53	V13	0.63	0.72	0.58	0.93
Var.77	V14	1.45	1.38	1.46	1.57
Kabacuara	V15	1.95	1.96	1.85	2.37
Cuarentino	V16	1.48	1.68	1.47	1.68
NEP 2	V17	1.54	1.56	1.35	1.72
Pronel	V18	1.50	1.18	1.55	1.66
Ocop 9X	V19	0.66	1.24	0.88	1.48
Pop 412	V20	1.47	1.59	1.24	1.85
Verdon	V21	1.45	1.35	1.67	1.86
W.C. 1210	V22	0.70	0.63	0.67	0.71
W.C. 1509	V23	0.75	0.58	0.81	0.94
W.C. 1046	V24	0.96	0.96	0.98	1.09
Var.100	V25	0.75	0.76	0.72	0.72

Table 5.24.5 (Contd.)

Analysis of Variance Table

Source	df	SS	MS	F
Varieties	24	49.69	2.07	345.00**
Seasons/Inocula	3	5.52	1.84	30.6**
Varieties x Seasons/Inocula	72	16.99	0.24	40.0**
Error	200	1.3183	0.006	
Total	299			

**indicates significance at the 1% level

Table 5.24.6 Comparison of methionine content (mg. available methionine/g. dry bean) of the four Variety Trials using Ranking Numbers

Variety	No.	1st rains 1971	2nd rains 1971	1st rains 1972	2nd rains 1972
Cundin a Marca	V1	23=	17=	19=	21
Ecuador 66	V2	15=	15	15	18
Ecuador 68	V3	13	12	12	13
Ecuador 184	V4	14	25	17	14
Mexico 119	V5	15=	14	15=	15
Mexico 137	V6	23	20=	24	22
Mexico 142	V7	22	24	22	23
Mexico 184	V8	11	7	10	10=
Mexico 200	V9	10	11	11	10=
Sanilac	V10	2	2	1	2
Seaway	V11	9	23	7	5
F.M.52	V12	20=	16	25	16
F.M.53	V13	25	19	23	20
Var.77	V14	7=	6	6	9
Kabacuara	V15	1	1	2	1
Cuarentino	V16	5	3	5	7
NEP 2	V17	3	5	8	6
Pronel	V18	4	10	4	8
Ocop 9X	V19	20=	9	14	12
Pop 412	V20	6	4	9	4
Verdon	V21	7=	8	3	3
W.C. 1210	V22	19	20=	21	25
W.C. 1509	V23	17=	22	18	19
W.C. 1046	V24	12	13	13	17
Var.100	V25	17=	17=	19=	24

5.25 Discussion of methionine results

The analysis of variance tables (Tables 5.24.1 - 5.24.4) for each of the four variety trials shows that there are significant differences in methionine content between the varieties. The level of significance was 1%. The tables show that for each variety trial, there are no significant differences between replicates.

Tables 5.24.5 however, which shows comparisons of methionine content between the four variety trials, shows that there are significant differences

(i) between varieties and

(ii) between seasons and/or inocula

(iii) between varieties x seasons/inocula interaction.

In each case the level of significance is 1%.

In order to separate the seasons effects from the inocula effects, the readings for methionine content for each of the four Variety Trials were ranked (see Table 5.24.6). In this way, the inocula effect was removed and only the differences between seasons were then being considered. It can be seen that in general, the low methionine varieties remained low in all seasons, and the high methionine varieties remained high. Thus, there was no seasonal effect on methionine content.

5.3 Effect of different fertiliser levels on the protein and methionine content

5.31 Materials and methods

The varieties used were :- F.M.53

Mexico 142

Sanilac

Kabacuara

Each of these varieties were grown in four different levels of mixed fertiliser, plus a no fertiliser control, and were replicated three times. The different levels of fertiliser used were:-

1) Control - consisting of loam + peat + sand mixture but zero John Innes base.
2) John Innes compost 1
3) John Innes compost 2
4) John Innes compost 3
5) John Innes Compost 4

The John Innes Compost 1 was prepared using

a) 7 parts by bulk of medium loam (C_3 field at Kabanyolo)
b) 3 parts of bulk of peat (Kabanyolo swamp)
c) 2 parts by bulk coarse sand (builders' sand)
d) $\frac{1}{4}$lb. John Innes Base)
e) $\frac{3}{4}$oz. ground chalk) per bushel of the above mixture

The John Innes Compost 2 was prepared using components (a) (b) (c) plus

$\frac{1}{2}$lb. John Innes Base)
$1\frac{1}{2}$oz. ground chalk) per bushel of the above mixture (a)+(b)+(c)

The John Innes Compost 3 was prepared using components (a) (b) (c) plus

$\frac{3}{4}$lb. John Innes Base)
2$\frac{1}{4}$oz. ground chalk) per bushel of the mixture (a)+(b)+(c)

The John Innes Compost 4 was prepared using the components (a) (b) (c) plus

1lb. John Innes Base)
3 oz. ground chalk) per bushel of the mixture (a)+(b)+(c)

The John Innes Base was bought ready mixed. It consisted of:-

2 parts weight hoof and horn meal

2 parts by weight superphosphate

1 part by weight of sulphate of potash

When mixing the compost, the loam and peat were first sieved. The sieved loam was then spread out a few inches thick, and covered first with peat then sand and finally the fertiliser (see Fig. 5.31.1)

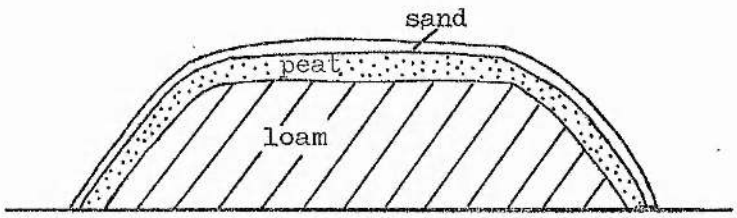


Fig. 5.31.1 Mixing the compost

The flat heap was then cut through in sections and turned with a spade until the whole pile had been turned. This procedure was then repeated two or three

times until the compost was thoroughly mixed. 18 lbs. of the compost was then put into each pot ready for planting.

The experimental plan is shown in Fig. 5.31.2.

Fig. 5.31.2 Experimental plan showing varieties and levels of fertiliser used

Varieties	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
F.M.53	0	0	0	1	1	1	2	2	2	3	3	3	4	4	4
Mexico 142	0	0	0	1	1	1	2	2	2	3	3	3	4	4	4
Sanilac	0	0	0	1	1	1	2	2	2	3	3	3	4	4	4
Kabacuara	0	0	0	1	1	1	2	2	2	3	3	3	4	4	4

Protein and methionine results only are considered in this chapter. For other characters see Appendix Table A5.31.1.

5.32 Protein Results

The mean protein results and analysis of variance are shown in Table 5.32.1. For more detailed protein results see Appendix Tables A5.32.1 - A5.32.4.

Table 5.32.1 The effect of different levels of fertiliser on the percentage crude protein content of four varieties

		Percentage crude protein
Variety	Fertiliser Level	Mean of 3 replicates
F.M.53	Control 0	20.6
F.M.53	1	22.8
F.M.53	2	23.3
F.M.53	3	22.2
F.M.53	4	19.9
Mexico 142	Control 0	24.4
Mexico 142	1	25.2
Mexico 142	2	25.3
Mexico 142	3	25.5
Mexico 142	4	24.2
Sanilac	Control 0	24.1
Sanilac	1	24.7
Sanilac	2	27.8
Sanilac	3	21.4
Sanilac	4	26.6
Kabacuara	Control 0	23.9
Kabacuara	1	24.0
Kabacuara	2	27.0
Kabacuara	3	20.7
Kabacuara	4	24.9

Analysis of Variance Table

Source	df	SS	MS	F
Varieties	3	99.80	33.27	11.88**
Treatment	4	77.09	19.27	6.88**
Varieties x treatment	12	87.67	7.31	2.61*
Error	30	83.85	2.80	
Total	59	348.41		

*indicates level of significance at 5%

**indicates level of significance at 1%

5.33 Discussion of protein results

The analyses of variance (Appendix Tables A5.32.1 - A5.32.4) showed that there were no significant differences in crude protein content with the varieties F.M.53 and Mexico 142 i.e. the indeterminate vine varieties. However, Sanilac (a determinate variety) and Kabacuara (an indeterminate bush variety) showed significant differences in protein content between treatments both of them at the 1% level - attributed clearly to a marked depression at level 3 of fertiliser.

Thus, with the indeterminate vine varieties F.M.53 and Mexico 142, the effect of fertility on the protein content was not so great as the effect with the determinate variety Sanilac and the indeterminate bush variety, Kabacuara. These differences of effects between the varieties could be explained by the fact that with the indeterminate vine varieties, the increases in fertiliser are used for vegetative growth and branching i.e. increases in fertiliser caused a greater degree of indeterminacy and to some extent an increase in yield. With the determinate variety there is vegetative growth to a lesser degree as the fertiliser is increased, so that perhaps the extra nitrogen is channelled into the seeds in the form of protein.

Table 5.32.1 shows the mean percentage crude protein content of each variety for each level of fertiliser and the analysis of variance below the

table shows that

(1) there were significant differences in protein content between the varieties at the 1% level.

(2) there were significant differences in protein content between the different levels of fertiliser treatment at the 1% level.

(3) there was a significant variety x treatment interaction attributable to differences in responsiveness.

Appendix Table A5.31.1 lists the seed yield in g. corresponding to the protein results in Table 5.32.1. The indeterminate vine varieties F.M.53 and Mexico 142 show gradual increases in yield with increasing levels of fertiliser corresponding to the steady levels in protein content. With the determinate variety Sanilac, and the indeterminate bush variety Kabacuara however, there are increases in yield to a maximum at fertiliser level 3 then a decrease in yield at fertiliser level 4. The maximum yields at fertiliser level 3 correspond to the low protein values at fertiliser level 3 indicating a negative correlation between seed yield and protein content. This seed yield and protein relationship is considered in more detail in Chapter 7.

5.34 Methionine results

The mean methionine results and the analysis of variance are shown in Table 5.34.1. For more detailed methionine results see Appendix Table A5.34.1. - A5.34.4.

Table 5.34.1 The effect of different levels of fertiliser on the methionine content (mg. methionine/g.dry bean) of four varieties

		mg.methionine/g.dry bean)
Variety	Fertiliser Level	Mean of 3 replicates
F.M.53	Control 0	0.65
F.M.53	1	0.62
F.M.53	2	0.60
F.M.53	3	0.63
F.M.53	4	0.61
Mexico 142	Control 0	0.66
Mexico 142	1	0.64
Mexico 142	2	0.67
Mexico 142	3	0.68
Mexico 142	4	0.64
Sanilac	Control 0	1.81
Sanilac	1	1.81
Sanilac	2	1.79
Sanilac	3	1.77
Sanilac	4	1.81
Kabacuara	Control 0	1.80
Kabacuara	1	1.77
Kabacuara	2	1.70
Kabacuara	3	1.76
Kabacuara	4	1.78

Table 5.34.1 (Contd.)

Analysis of Variance Table

Source	df	SS	MS	F	
Varieties	3	19.3666	6.4555	2511.86**	
Treatment	4	0.0094	0.00235	0.914	n.s.
Varieties x Treatment	12	0.0296	0.00246	0.957	n.s.
Error	30	0.1029	0.00257		
Total	59	19.5085			

**indicates significance at the 1% level

n.s. indicates non significance

5.35 Discussion of Methionine Results

The analysis of variance in Table 5.34.1 showed that.

(1) there were significant differences in methionine between varieties at the 1% level.

(2) there were no significant differences in methionine between different fertiliser level treatments.

(3) there was no significant variety x fertiliser treatment interaction.

Thus increasing levels of fertiliser treatment had no effect on methionine content of the seed.

CHAPTER 6 GENETICAL STUDIES ON THE PROTEIN CONTENT OF THE BEANS USING F_1, F_2 AND F_3 GENERATIONS OF A 7 x 7 NON RECIPROCAL DIALLEL OF SELECTED CONTRASTING PARENTS

6.1 Introduction

A quantitative genetical approach to the study of protein inheritance has been adopted on the assumption that protein content is under genetical control and is not inherited in a simple Mendelian fashion. The polygenic model of many genes of cumulative effect that may either act independently (additive effects or in a way dependent upon other genes (non-additive interactions including dominance and epistasis) is one that has been widely applied to problems of quantitative inheritance and for which standard mathematical analyses are available. An analysis of a diallel set of crosses between homozygous lines, their F_1 recombinants and the respective F_2 progeny following selfing has been adopted for this study. One of the conclusions that can usually be made from such a study is the adequacy or otherwise of the model chosen. Various analyses of diallel crossing systems have been developed by Mather (1949); Jinks and Hayman (1953); Hayman (1954(a), 1954(b)); Jinks (1954, 1956) and others.

If the model behind the analysis of Jinks and Hayman is proved suitable by tests with the data obtained, then this analysis can provide information on the average additive effects of the genes involved, the effects of heterozygosity and the average effects of the dominance of the genes in the parents. In addition other parameters can be estimated namely, the dominance relationship, the number of effective factors controlling a character and the frequency of genes in each chosen parent which increase or decrease the mean of the character. There are sufficient statistics available

from a diallel table to provide estimates of these genetic components. The square tables of results from a diallel set of crosses lend themselves to a variety of alternative analyses of variance which can be used to test the significance of some of the genetical components of variation and also the validity of some of the subsidiary assumptions underlying the simple model, such as the absence of differences between reciprocal crosses. Analyses have been described which allow for every conceivable variation in experimental design including the presence and absence of parental means and reciprocal crosses (e.g. Yates, 1947; Hayman, 1954(a); Griffing, 1956; Jones, 1965) and differing relative degrees of replication of diagonal (parental) and off-diagonal (F_1) entries in the diallel table (Jones, 1965). There is a corresponding range of alternative methods of partitioning the total variation (Jinks, 1954); Hayman, 1954(b); Griffing, 1956; Jinks and Mather, 1955; Henderson, 1952) and methods of deriving the variance components from the mean squares according to whether maternal or reciprocal effects are present or not (Griffing, 1956; Wearden, 1964) and whether the parental lines are a fixed sample or a random sample of a population of inbred lines (Griffing 1956); Wearden, 1964; Hayman, 1960).

The general requirements of any analysis of variance of a diallel table are that it provides appropriate tests of significance of the principle genetic components, namely additive and non-additive effects, irrespective of whether there are reciprocal differences among the progeny families, and provides a test for the presence of

the latter. The diallel crossing programme therefore allows a genetic analysis to be carried out after one or more generations and provides tests of the adequacy of the model.

Many investigators have applied this Jinks and Hayman analysis in attempts to aid the practical assessment of parental strains to employ in breeding programmes for example, the work of Allard (1956) on lima beans, the work of Leffel and Hanson (1961) on soyabeans and the work of Whitehouse Thompson and Ribeiro (1958) on wheat. But, in few cases, if any, have parents actually been chosen for a subsequent practical breeding programme that would otherwise have been overlooked.

The diallel cross analysis of Jinks and Hayman (1953) was thought to be worth using for the investigation of the genetic control of the protein content of the varieties of Phaseolus vulgaris. It was decided that seven parents should be used taking into account practical constraints on the time and labour required and the space available for cultivation of the hybrid progeny. The varieties selected from the collection were Mexico 142, Sanilac, F.M. 52 , Kabacuara, Pop 412, W.C. 1210, NEP2 (see Plates 1-7). These varieties were selected after a careful study of their origin (see Table 6.1.1) and their various phenotypically desirable characters as shown in Table 6.1.2. These varieties were specifically chosen and do not represent a random sample of all white haricot bean varieties. Therefore, inferences made from the data apply only to the varieties and crosses studied.

Table 6.1.1 Origins of the parents chosen for the diallel cross

Mexico 142 Origin:- Mexico. Accepted as a commercial variety in East Africa (and also under the names Ethiopia 10 and Tengeru 16).

Sanilac (Tengeru 36) Origin:- Michelite was treated with 100KV X-rays (Down and Anderson, 1956). From the populations which resulted, a dwarf mutant (determinate) was located which was then backcrossed to the parental genotype Michelite for three generations. Three lines were selected from these backcrosses, that were determinate and substantially earlier in maturity than Michelite. These numbers were 8474, 8479, and 8375. Sanilac's pedigree involves two of these Michelite mutants and other navy beans (Anderson, Down and Whitford 1960). The full pedigree is:-

/(Robust and Crawford x Emerson 847)x(8374)/x(BCF 8479 x Emerson 53)
11

F.M. 52 Origin:- It was developed by the Ferry Morse seed company in California. It arose from crosses made with California small white x Michelite and Mosaic Resistant U.I. (University of Idaho) No. 2. It is resistant to rust and it is probable that this resistance was derived from the Idaho breeding line.

Kabacuara Origin:- It is a selection from the Kabanyolo breeding material in Uganda. The single parent plant appeared spontaneously within a population of Cuarentino from Rwanda, probably as a result of an illegitimate outer-crossing with a pea bean. It was selected by Leakey and subsequently reselected through several generations by mass selection (Leakey, 1969).

Pop412 Origin:- It resulted from the crosses /Prélude x (Processor x Cornell 49-242)/ made by Bannerot and his associates at Versailles (Leakey, 1971). It is homozygous for resistance to anthracnose based on the 'are' gene. It also has high protein content and high arginine content near to that of the pea (Leakey and Stabursvik, 1971).

W.C. 1210 Origin:- Chile. It is an extremely vigorous navy bean but with seeds well over pea bean size. It was screened at Kabanyolo from a study of a large bean collection assembled by USDA/USAID in the Iran - Regional Improvement Programme.

NEP 2 Origin:- Developed by Moh (1969 and 1971) in Costa Rica. The black seeds of San Fernando were treated with various concentrations of E.M.S. and X-rays. The hypocotyls of the M_2 seed were green or purple. The green hypocotyl plants were selected as plants likely to have white seeds. Five mutants were selected, one of these being designated NEP2. It appears to resemble the parental San Fernando in all other respects than the absence of anthocyanin production.

Table 6.1.2 Characters of the seven varieties chosen for the diallel cross

Variety	Habit	Rust 0-5	Seed Colour	Seed Size mm.	100 seed wt. in g.	1 Seed yield g./plant	No. of seeds per plant	2 % crude protein (D.W.B)	3 Protein yield g./plant	4 Mg.Methionine /g.bean
Mexico 142	IV	1	W	6	16.4	11.5	70	26.2	2.7	0.67
Sanilac	D	5	W	5	14.4	5.0	35	29.0	1.3	1.86
F.M.52	IV	2	W	7	17.6	10.7	61	21.0	2.1	0.66
Kabacuara	IB	0	W	7	21.9	23.0	105	26.0	5.4	1.95
Pop 412	D	5	W	8	22.4	9.1	41	31.4	2.2	1.47
W.C.1210	IV	0	W	8	23.0	14.5	63	27.0	3.5	0.70
NEP 2	IB	0	W	7	18.8	21.3	113	26.5	5.1	1.54

KEY
IV = Indeterminate vine
IB = Indeterminate bush
D = Determinate
W = White

NOTES
1. Based on 21 single plants
2. Based on duplicate analyses of 21 single plants
3. Based on the results of 1 and 2 (W.W.B)
4. Based on duplicate analyses of 21 single plants

These varieties were grown in consecutive seasons in the greenhouse to check that they were pure lines i.e. to make sure that there was no apparent genetic variation due to accidental intercrossing. This was necessary to meet the requirement of complete parental homozygosity which is one of the assumptions of the genetic model of the diallel cross analysis of Jinks and Hayman (1953). The full assumptions of this analysis are listed below.

(a) parental homozygosity

(b) normal diploid segregation

(c) no difference between reciprocal crosses

(d) no multiple alleles

(e) no linkage

(f) no interallelic interaction

These assumptions must first be tested. No extra analysis can be undertaken if the genetical system fails to satisfy these assumptions. If the system does fail to satisfy these assumptions, then it will be detected by the heterogeneity of (Wr-Vr) where Wr = covariance of the r^{th} array and Vr = variance of the r^{th} array. Two tests for the heterogeneity of Wr-Vr are available.

(1) When the experiment is replicated, the variance of Wr-Vr may be analysed for line and block differences. A significant line effect indicates failure of the assumptions.

(2) A test which is useful when the experiment is not replicated depends on the Wr, Vr graph. This is not a line of unit slope if Wr-Vr varies, and hence a failure of the assumptions.

After the selection of the varieties for investigation and the testing of the assumptions of the diallel cross and its analysis, the present study was carried out to investigate the inheritance of crude protein content in Phaseolus vulgaris. The findings are discussed in relation to improvement of the bean crop.

6.2 Materials and methods

A hybridisation programme was set up to produce the diallel crosses required. Seven varieties were selected on the basis of yield, habit, rust resistance, seed size shape and colour and the protein and methionine content (see Table 6.1.2.) These assumed pure lines were then crossed with each other in each combination but omitting reciprocals. The hybridisation technique which was used is described below.

Hybridisation technique

A flower, (in the bud stage of development) of the famale parent was first emasculated - the anthers were removed with fine tweezers. A stigma, laden with fresh pollen was then removed from a fully opened fresh flower of the male parent. This laden stigma was then hooked onto the stigma of the emasculated bud of the female parent. The bud was then labelled. The percent of successful crosses was usually in the range of 50 - 60%. Four hybrid seeds of each cross were obtained as shown in Table 6.2.1. (no reciprocals)

Table 6.2.1 Diallel cross table showing the number of F_o hybrid seed obtained

Variety	Mexico 142	Sanilac	F.M.52	Kabacuara	Pop412	W.C.1210	NEP2
Mexico 142	X	4	4	4	4	4	4
Sanilac		X	4	4	4	4	4
F.M.52			X	4	4	4	4
Kabacuara				X	4	4	4
Pop 412					X	4	4
W.C. 1210						X	4
NEP2							X

The future generations were obtained as shown in Table 6.2.2. The nomenclature for each generation is based entirely as if maternal effects were 100%. The nomenclature is shown in the form of a diagram below.

Table 6.2.2. The generations obtained for analysis of percentage crude protein

Fo hybrid single seed* → Female Parent*

Male Parent*

Fo hybrid single seed → F_1 hybrid Plant*

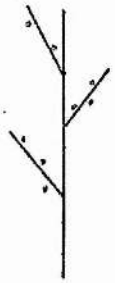
Fo hybrid single seed → F_1 hybrid plant → F_2 generation*

Fo hybrid single seed → F_1 hybrid plant → F_2 generation → F_3 generation*

*indicates the generations to be analysed.

All generations could therefore be grown in the same season and subsequently analysed for crude protein.

NOMENCLATURE OF ALL GENERATIONS USED IN THE TEXT

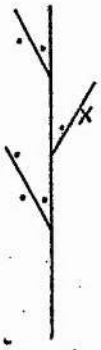


• Po seed, cotyledon and embryonic axis have the same genotype as the parent.

Parental Plant Selfed

Treated in the text as Po seed

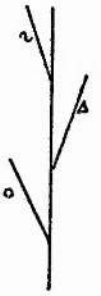
Po seed, cotyledon and embryonic axis have the same genotype as the parent



X Fo seed from a crossed flower - the pod contains seeds with maternal testa only but the cotyledons and embryonic axis have F_1 genotype.

Treated in the text as Fo seed

Parental plant carrying selfed seed and hybrid seed

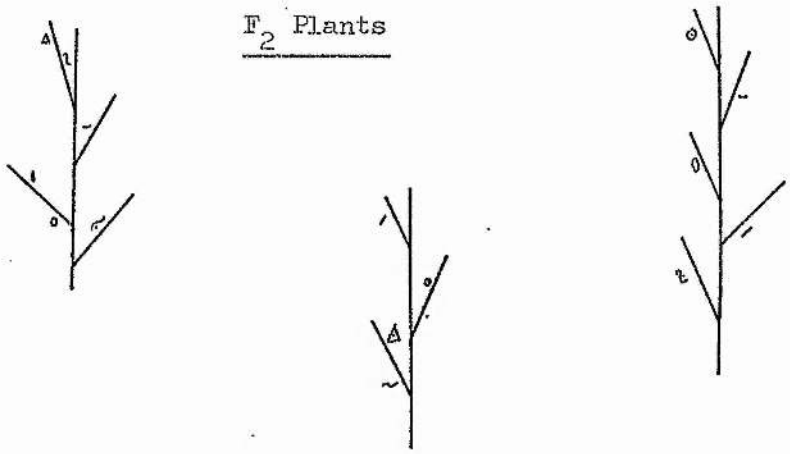


F_1 Plant

Treated in the text as if "F_1 seed" i.e. convention used that F_1 seeds means seeds on F_1 plants.

All seeds are different genetically from each other - only similarity due to (1) testa (2) maternal effect. The seeds contain the cotyledons and embryonic axis of F_2 genotype but are the seeds on the F_1 hybrid plant (each seed is unique)

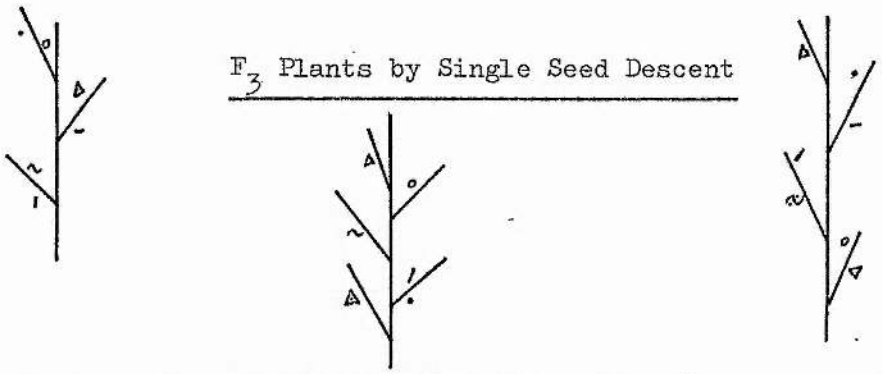
F_2 Plants



Each plant is now genetically different. Also each seed within each plant is different. The seeds contain the cotyledons and embryonic axis of potential F_3 genotypes but are the seeds on the F_2 plant.

Treated in the text as if "F_2 seed" i.e. convention used that F_2 seeds means seeds on F_2 plants.

F_3 Plants by Single Seed Descent



Each plant is again genetically different. Also each seed within each plant is different. The seeds contain the cotyledons and embryonic axis of potential F_4 genotypes but are the seeds on the F_3 plants.

Treated in the text as if "F_3 seed" i.e. convention used that F_3 seeds means seeds on F_3 plants.

In the field

The seeds from each generation were planted in progeny rows with an inter-row spacing of 2 ft. and an intra-row of 6 ins. The female parents, male parents, F_1 hybrid plants, F_2 hybrid plants, F_3 hybrid plants of each cross were grown in the first rains of 1973. The F_3 hybrid generation was derived from single seed descent from the entire F_2 population obtained i.e. one seed from each F_2 plant of each cross was planted to produce the equivalent number of F_3 plants. This does not eliminate drift but eliminates some bias. In fact, three seeds from a single pod from a low node on an F_2 plant were planted. These were then thinned to one plant to provide corresponding numbers of F_2 and F_3 plants (but F_3 plants were derived from a different set of F_2 plants not the same set).

In the experimental design twenty one blocks were balanced so that each block contained

(1) 3 plants of each of the seven parents

(2) 1 F_1 plant

(3) $\frac{1}{21}$ of the F_2 plants of each of cross 1,2,3.....21.

(4) $\frac{1}{21}$ of the F_3 plants of each of cross 1,2,3.....21.

Each single plant was scored for habit, rust reaction, total seed yield and the percentage crude protein of the seeds.

The design could then be analysed to see if any of the variance within or between blocks should be applied to error. The parents which were replicated three times within each block and twenty one times between blocks were used to obtain an estimate of "environmental" variance (inclusive of genetic effects of the segregants due to drift).

Analytical technique

All nitrogen data used in the analyses was carried out on the Technicon nitrogen autoanalyser. The method of analysis is described in section 3.22of chapter 3. The sampling procedure for seeds within plants was as for a single homozygous plant i.e. the seeds from approximately three pods from low, intermediate, and high nodes. These were then bulked, dried, milled and analysed.

6.3 Protein Results

The percentage crude protein results for the parents are shown in Table 6.3.1. Each value in each block is the value obtained on a mean of 2 analytical replications of a sample prepared from 25 seeds sampled from a single plant.

The standard errors of the means and the coefficient of variation values are shown to be homogeneous which therefore validates the Analysis of Variance (also shown in Table 6.3.1).

Table 6.3.1 Percentage crude protein results for the parents grown in the 2nd Rains 1973

	Block No.																					Mean	s.e.	C.V.
Variety	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
Mexico 142	24.3	23.9	24.2	24.6	23.7	24.9	24.4	26.1	24.3	24.1	26.3	23.1	23.7	25.1	25.2	26.3	24.6	25.8	25.5	23.4	23.1	24.6	0.21	4.00
Sanilac	29.6	27.4	28.3	27.6	27.6	29.8	29.4	29.2	28.0	29.8	28.6	30.8	31.0	29.2	28.6	29.8	29.2	28.6	30.4	29.0	28.6	29.1	0.22	3.49
F.M.52	22.4	19.9	20.6	22.0	19.2	22.8	21.2	21.7	21.1	19.9	20.6	22.9	21.4	20.6	20.0	21.0	21.6	20.7	21.6	21.1	21.6	21.1	0.20	4.37
Kabacuara	27.8	27.4	25.9	26.5	28.2	26.1	27.8	26.4	26.8	26.8	25.8	27.2	28.0	29.3	26.9	28.2	26.9	26.0	26.0	27.8	27.7	27.1	0.19	3.27
Pop 412	30.5	27.1	32.2	30.4	29.2	30.5	29.6	27.4	29.7	30.5	29.9	31.6	31.6	27.4	31.6	29.8	31.3	28.6	29.4	30.7	30.2	30.0	0.44	6.80
W.C. 1210	24.8	24.2	23.9	24.4	24.9	24.2	25.2	24.7	26.1	23.2	26.4	25.7	24.4	26.9	26.7	24.0	26.9	25.7	24.4	24.7	26.0	25.1	0.25	4.59
NEP 2	27.8	25.5	26.1	25.6	25.0	25.5	27.4	25.3	26.5	25.0	27.9	28.9	26.9	27.0	26.2	25.0	26.4	27.0	27.3	25.6	27.3	26.4	0.26	4.57

Each value in each block is the value obtained on a mean of 2 analytical replications of a sample prepared from 25 seeds sampled from a single plant.

Table 6.3.1 (Contd.)

Analysis of Variance Table

Source	df	SS	MS	F	
Varieties	6	1105.84	184.31	169.66**	
Blocks	20	33.65	1.68	1.55	n.s.
Error	120	130.36	1.09		
Total	146	1269.85			

**statistically significant at the 1% level

n.s. indicates non significance

The analysis of variance in Table 6.3.1 shows that

(1) there were significant differences in protein content between the varieties at the 1% level but

(2) there were no significant differences in protein content of the same variety grown in the different blocks.

On this account no possible variety x block (genotype x environment) interaction was further investigated.

Therefore, there was no significant differences in "environment" and so all the results from each block could later be pooled together for the purpose of the diallel analysis.

The results of Table 6.3.2 show that in general, the crude protein range of the parents was about 3.5 with the exception of Pop 412 at 5.1.

The protein results for the Fo hybrid single seed borne on the maternal plant and also the protein results for the seeds borne on the F_1 hybrid plants, are listed in Table 6.3.3.

For the Fo hybrid single seed there was no duplication of samples. The whole seed was dried, weighed, digested and subsequently analysed. Therefore there would be some unavoidable analytical experimental error involved.

Table 6.3.2 Protein range of parents

Parent	Mean % crude protein of 21 single plants	Crude protein range	Span
Mexico 142	24.6	23.1-26.3	3.2
Sanilac	29.1	27.4-31.0	3.6
F.M.52	21.1	19.2-22.9	3.7
Kabacuara	27.1	25.8-29.3	3.5
Pop 412	30.0	27.1-32.2	5.1
W.C. 1210	25.1	23.2-26.9	3.7
NEP 2	26.4	25.0-28.9	3.9

Table 6.3.3 Protein results of the parents, mid parent value, F_o hybrid single seed borne on the maternal plant and the seeds borne on the F_1 hybrid plant

Cross No.	♀ Parent		♂ Parent		Mid parent value	F_o single seed	Seeds on F_1 plant
1	Mexico 142	24.6	Sanilac	29.1	26.9	25.2	26.6
2	Mexico 142	24.6	F.M.52	21.1	22.9	24.3	20.6
3	Mexico 142	24.6	Kabacuara	27.1	25.9	25.2	26.3
4	Mexico 142	24.6	Pop 412	30.0	27.3	24.6	25.8
5	Mexico 142	24.6	W.C. 1210	25.1	24.9	25.7	24.8
6	Mexico 142	24.6	NEP 2	26.4	25.5	24.2	25.2
7	Sanilac	29.1	F.M.52	21.1	25.1	28.6	24.8
8	Sanilac	29.1	Kabacuara	27.1	28.1	29.8	28.0
9	Sanilac	29.1	Pop 412	30.0	29.6	29.1	29.8
10	Sanilac	29.1	W.C.1210	25.1	27.1	29.6	26.4
11	Sanilac	29.1	NEP 2	26.4	27.8	29.0	27.6
12	F.M.52	21.1	Kabacuara	27.1	24.1	21.5	23.6
13	F.M.52	21.1	Pop 412	30.0	25.6	21.3	22.5
14	F.M.52	21.1	W.C.1210	25.1	23.1	21.6	23.4
15	F.M.52	21.1	NEP2	26.4	23.8	21.0	21.2
16	Kabacuara	27.1	Pop 412	30.0	28.6	28.2	25.8
17	Kabacuara	27.1	W.C.1210	25.1	26.1	27.0	24.2
18	Kabacuara	27.1	NEP2	26.4	26.8	27.6	25.6
19	Pop 412	30.0	W.C.1210	25.1	27.6	26.6	25.7
20	Pop 412	30.0	NEP 2	26.4	28.2	31.4	26.5
21	W.C.1210	25.1	NEP 2	26.4	25.8	26.1	24.4

The frequency distributions of percent crude protein for the generations of the 21 crosses of the diallel are shown in Tables 6.3.4 - 6.3.24

Table 6.3.4 Frequency distribution of percent crude protein for the generations of cross 1, Mexico 142 x Sanilac

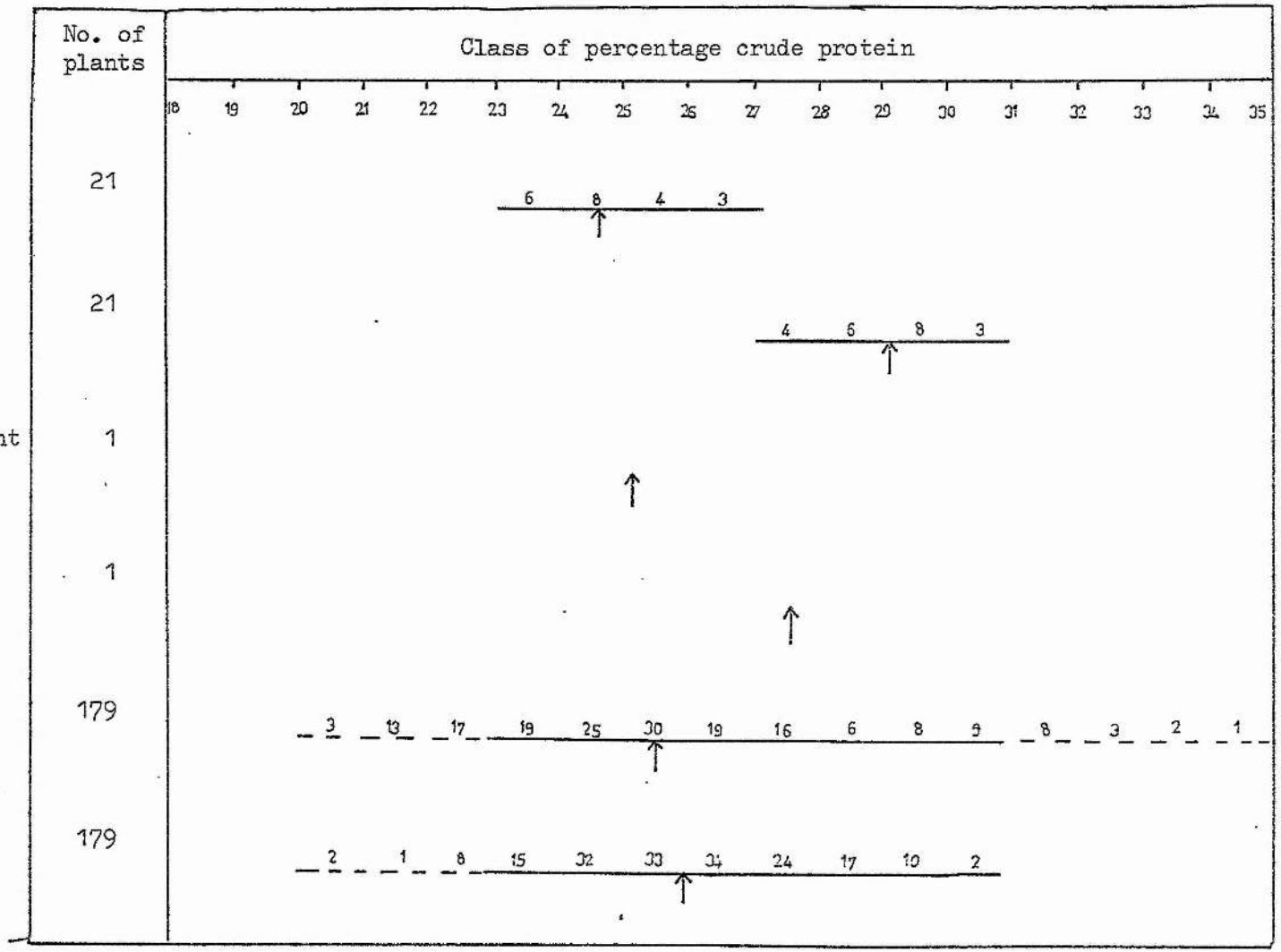


Table 6.3.5 Frequency distribution of percent crude protein for the generations of cross 2, Mexico 142 x F.M.52

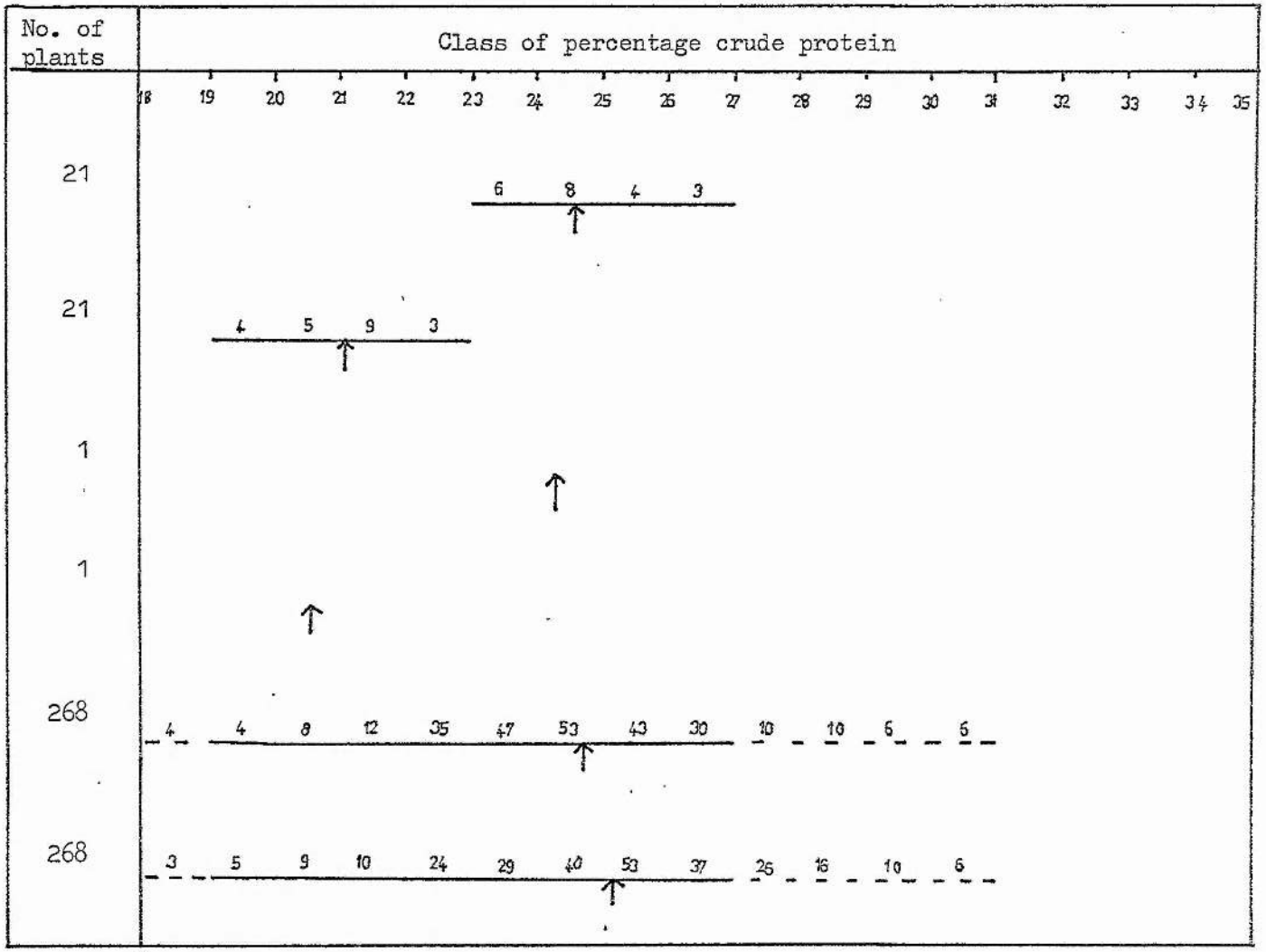


Table 6.3.6 Frequency distribution of percent crude protein for the generations of cross 3, Mexico 142 x Kabacuara

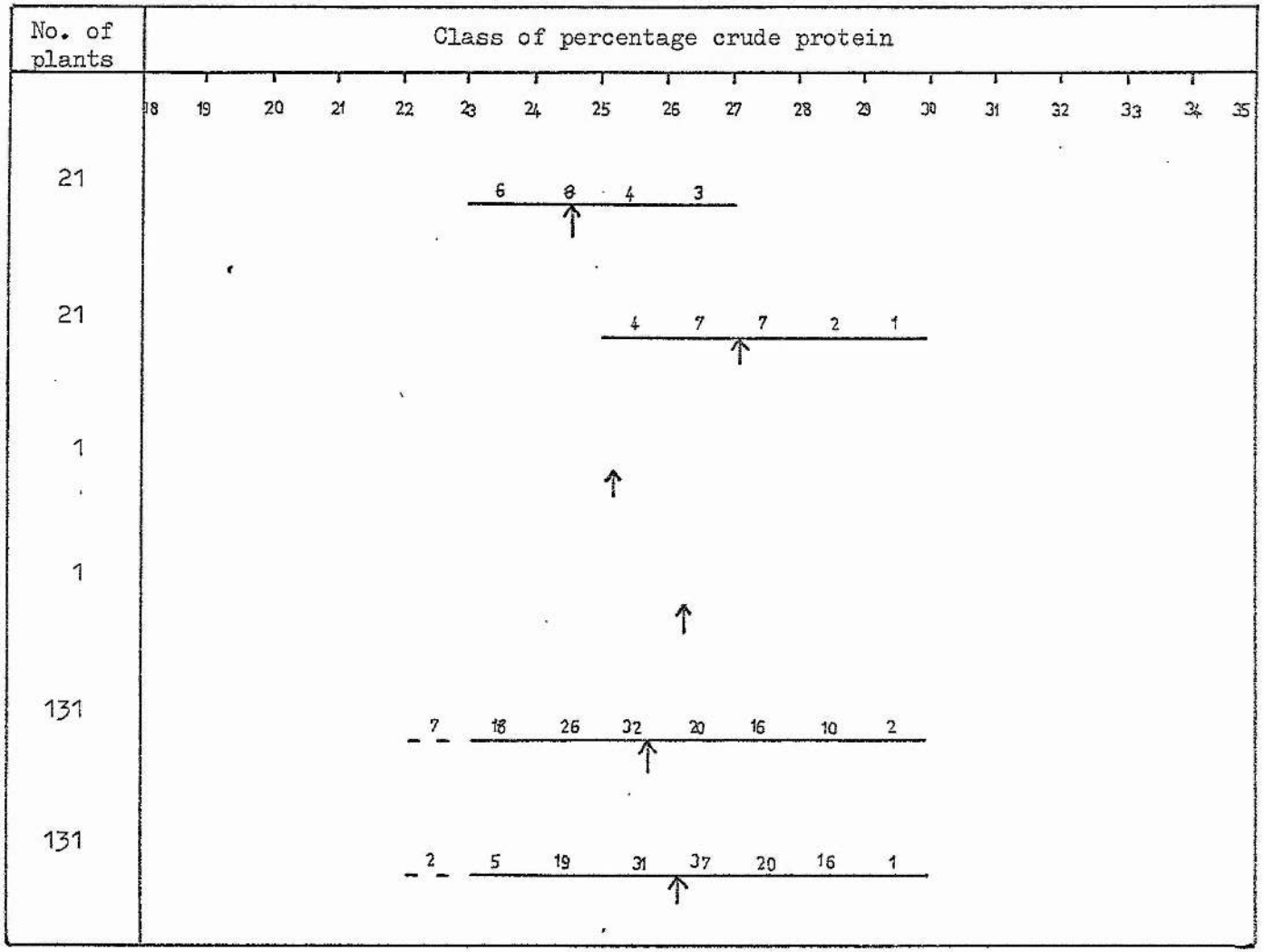


Table 6.3.7 Frequency distribution of percent crude protein for the generations of cross 4, Mexico 142 x Pop 412

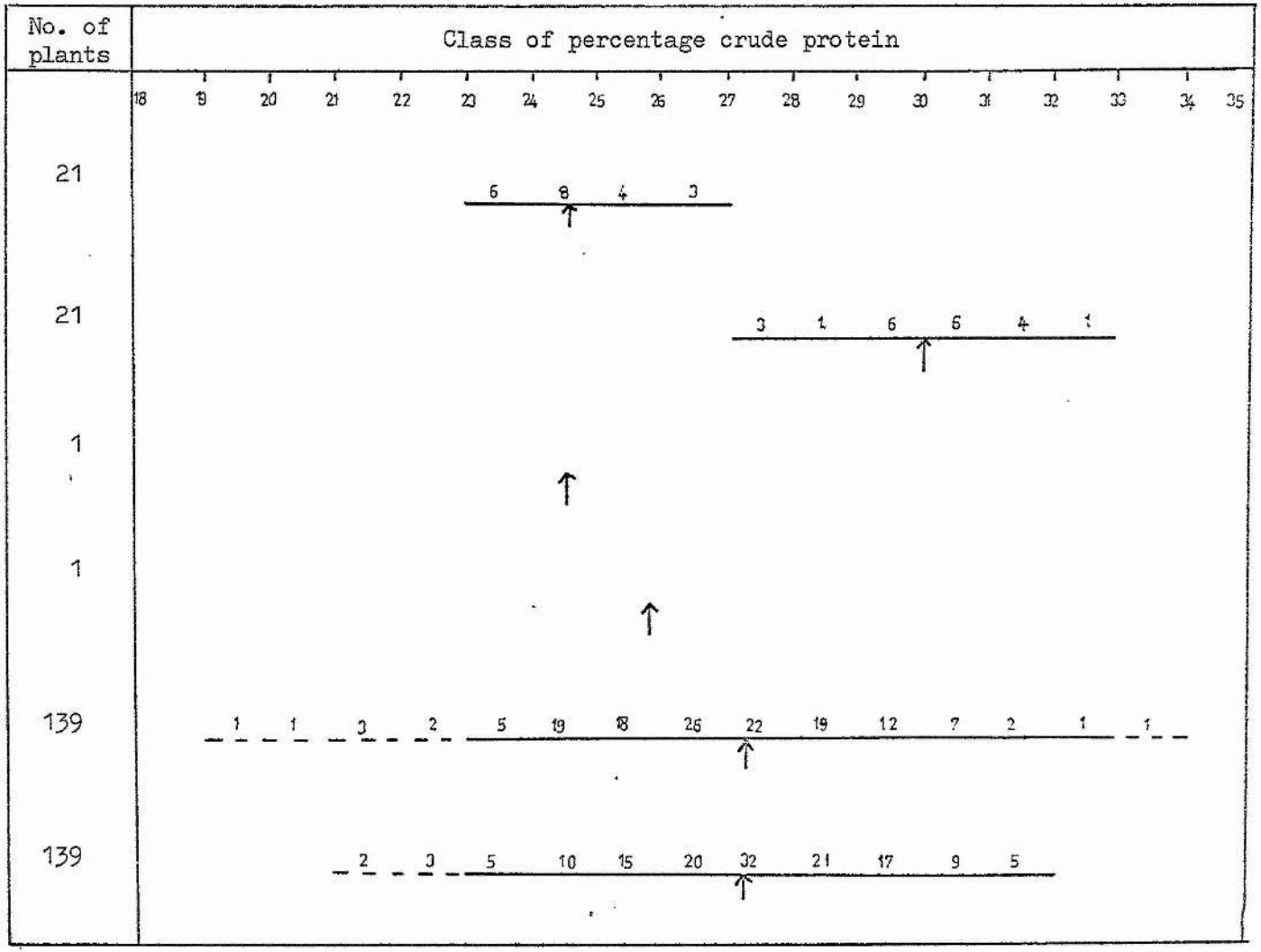


Table 6.3.8 Frequency distribution of percent crude protein for the generations of cross 5, Mexico 142 x W.C. 1210

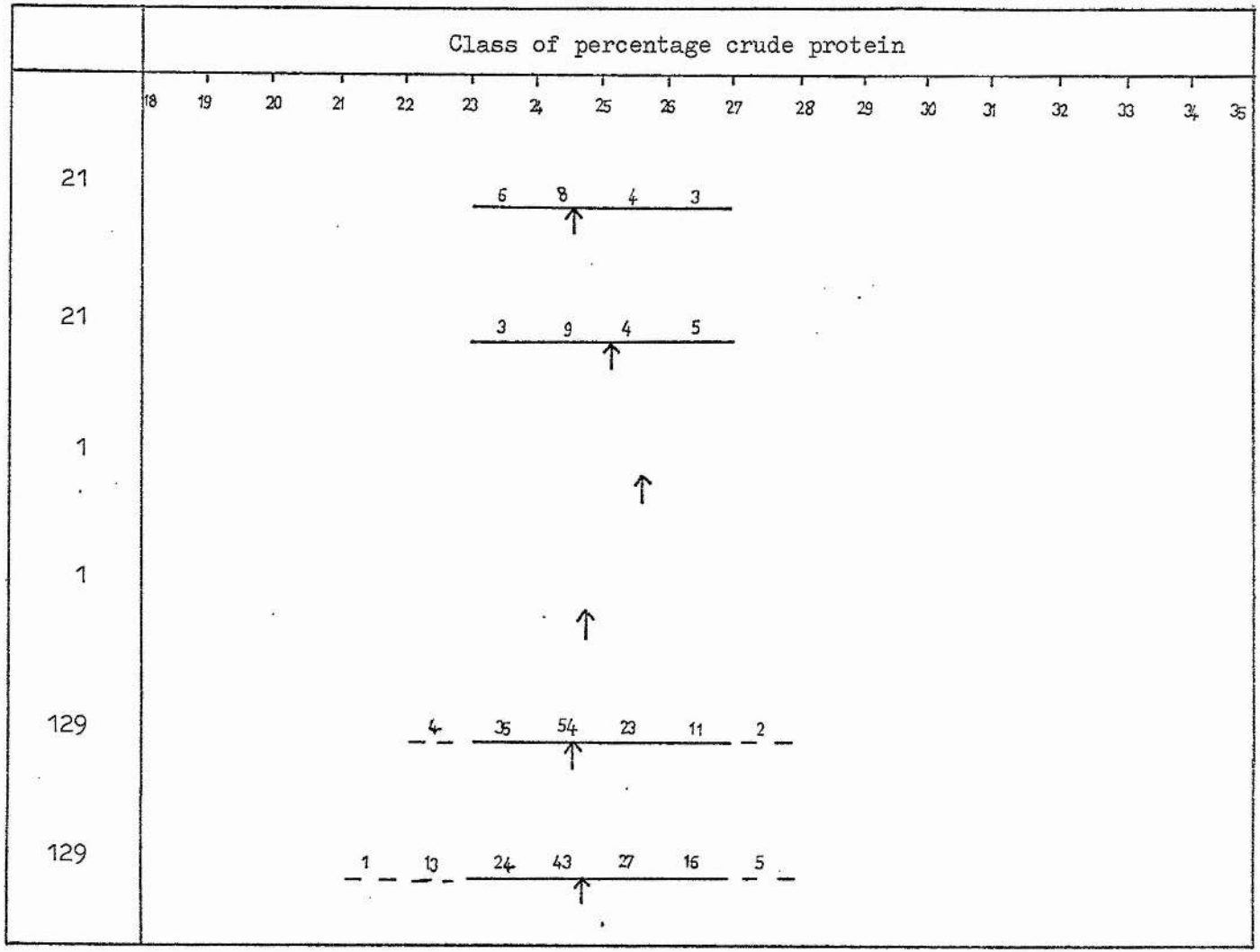


Table 6.3.9 Frequency distribution of percent crude protein for the generations of cross 6, Mexico 142 x NEP 2

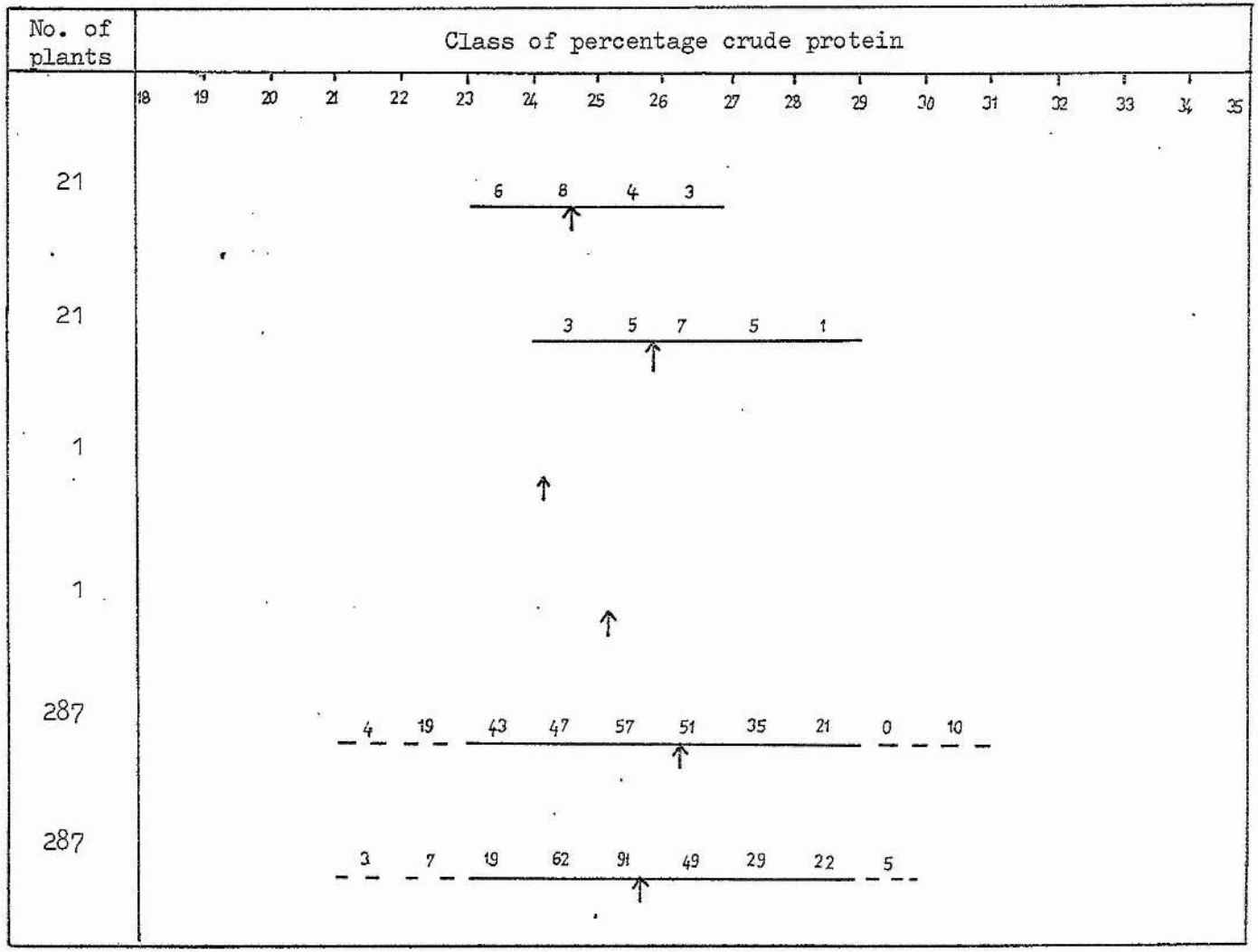
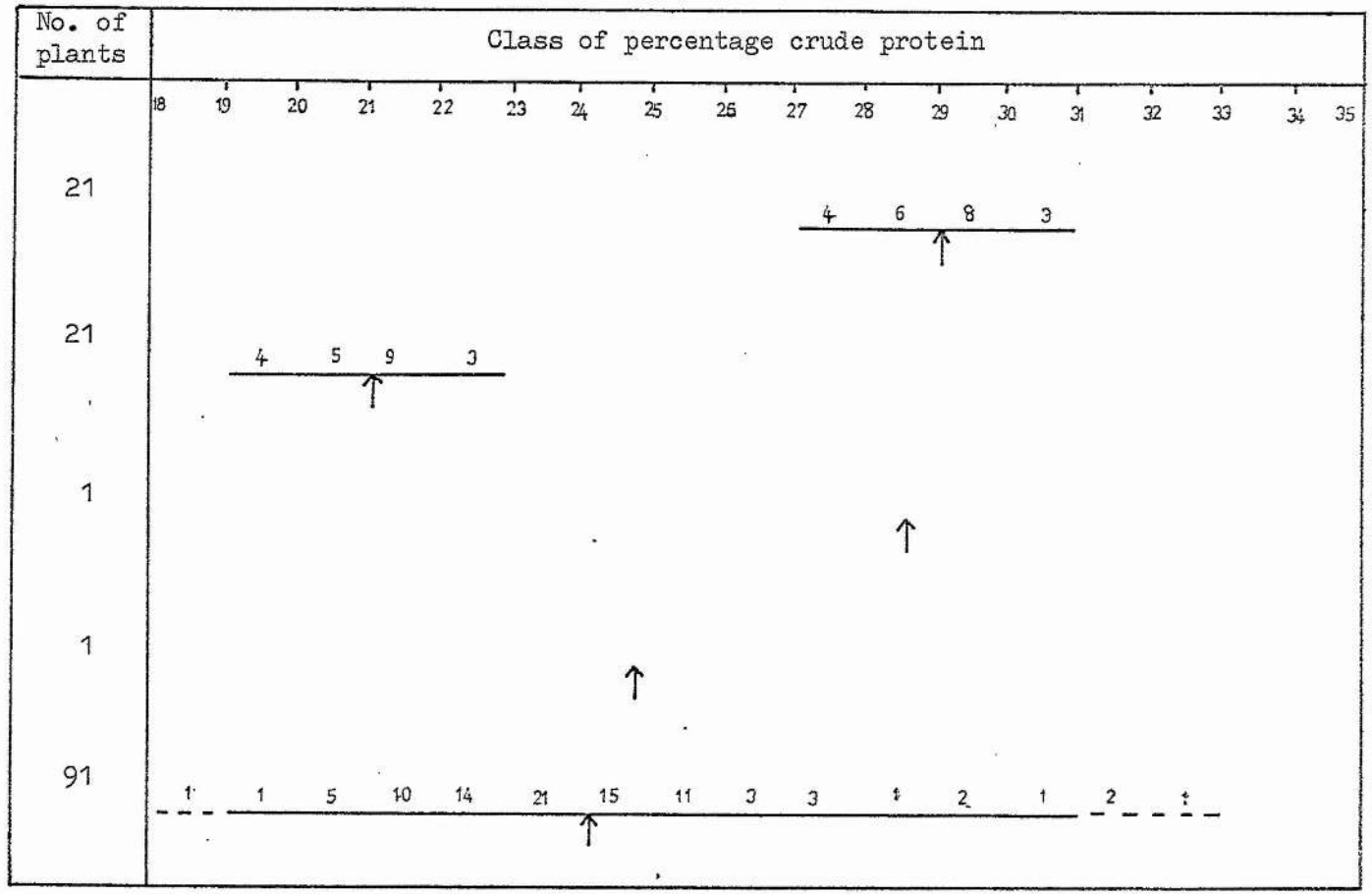
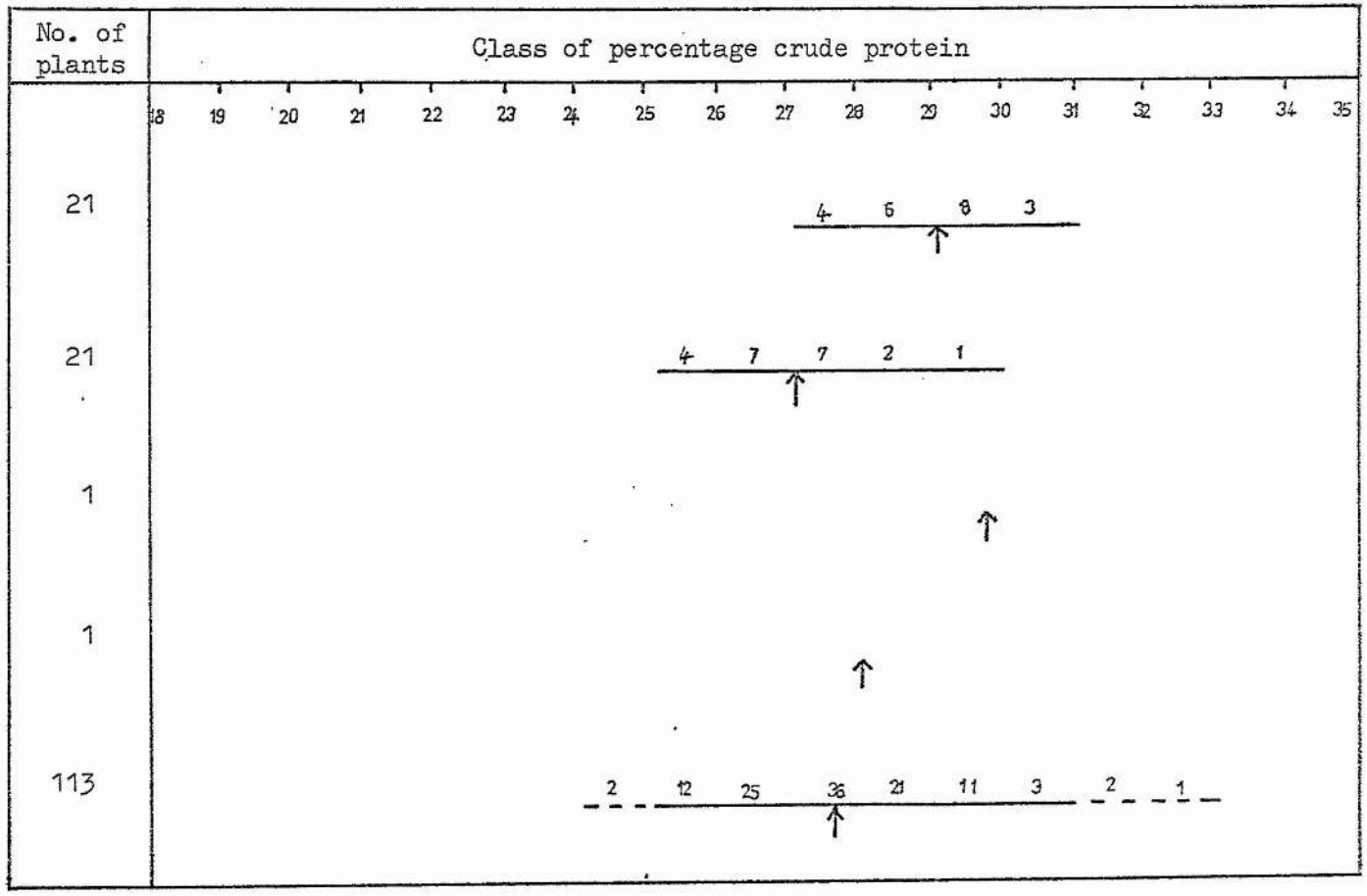


Table 6.3.10 Frequency distribution of percent crude protein for the generations of cross 7, Sanilac x F.M.52



↑ indicates the mean

Table 6.3.11 Frequency distribution of percent crude protein for the generations of cross 8, Sanilac x Kabacuara



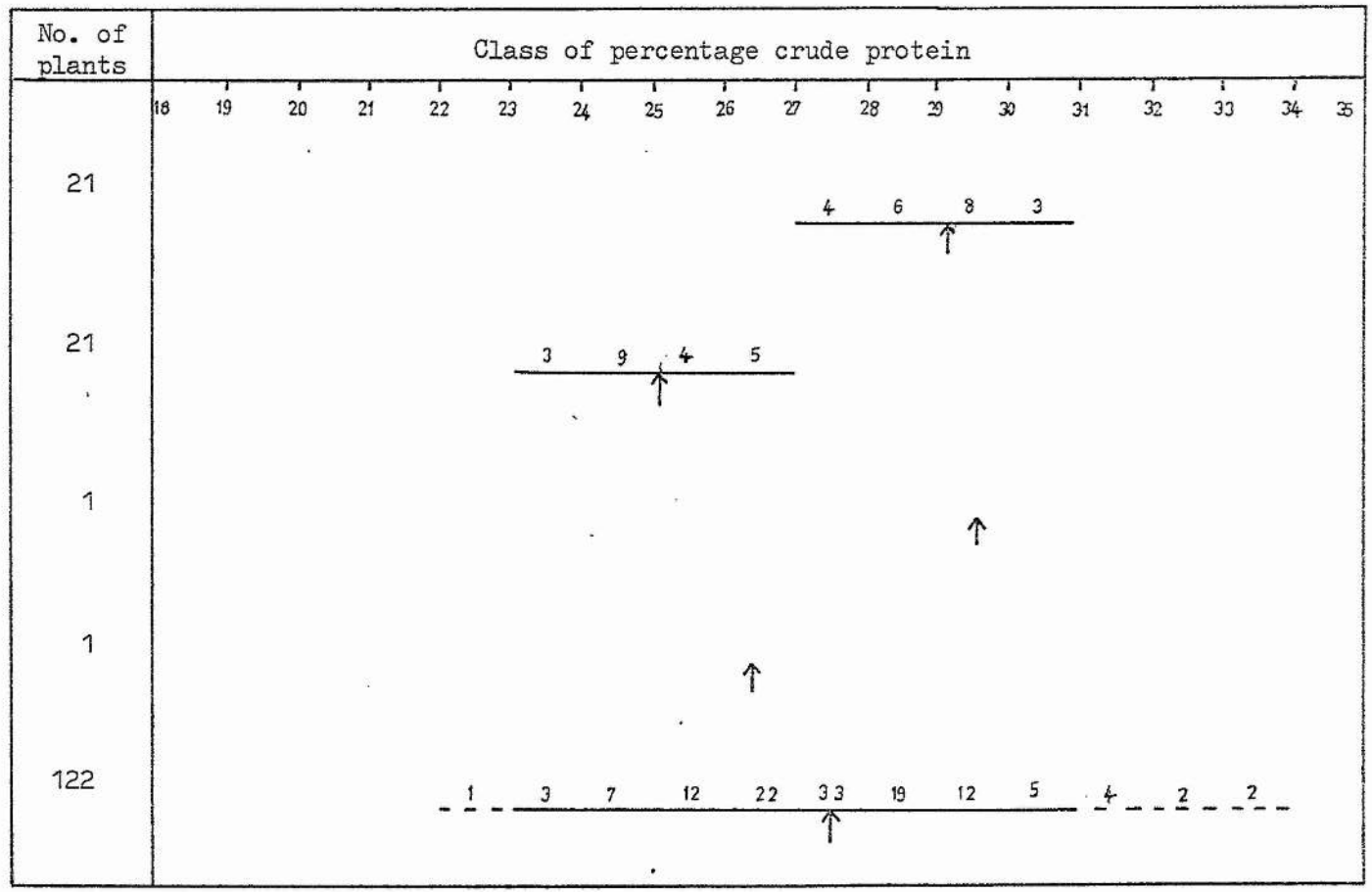
↑ indicates the mean

Table 6.3.12 Frequency distribution of percent crude protein for the generations of cross 9, Sanilac x Pop 412

	No. of plants	Class of percentage crude protein
		18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
♀ parent, Sanilac	21	4 6 8 3
♂ parent, Pop 412	21	3 1 6 6 4 1
Fo hybrid seed on maternal plant	1	
F_1 hybrid plant	1	
F_2 generation plants	165	1 7 10 22 32 44 26 15 6 2

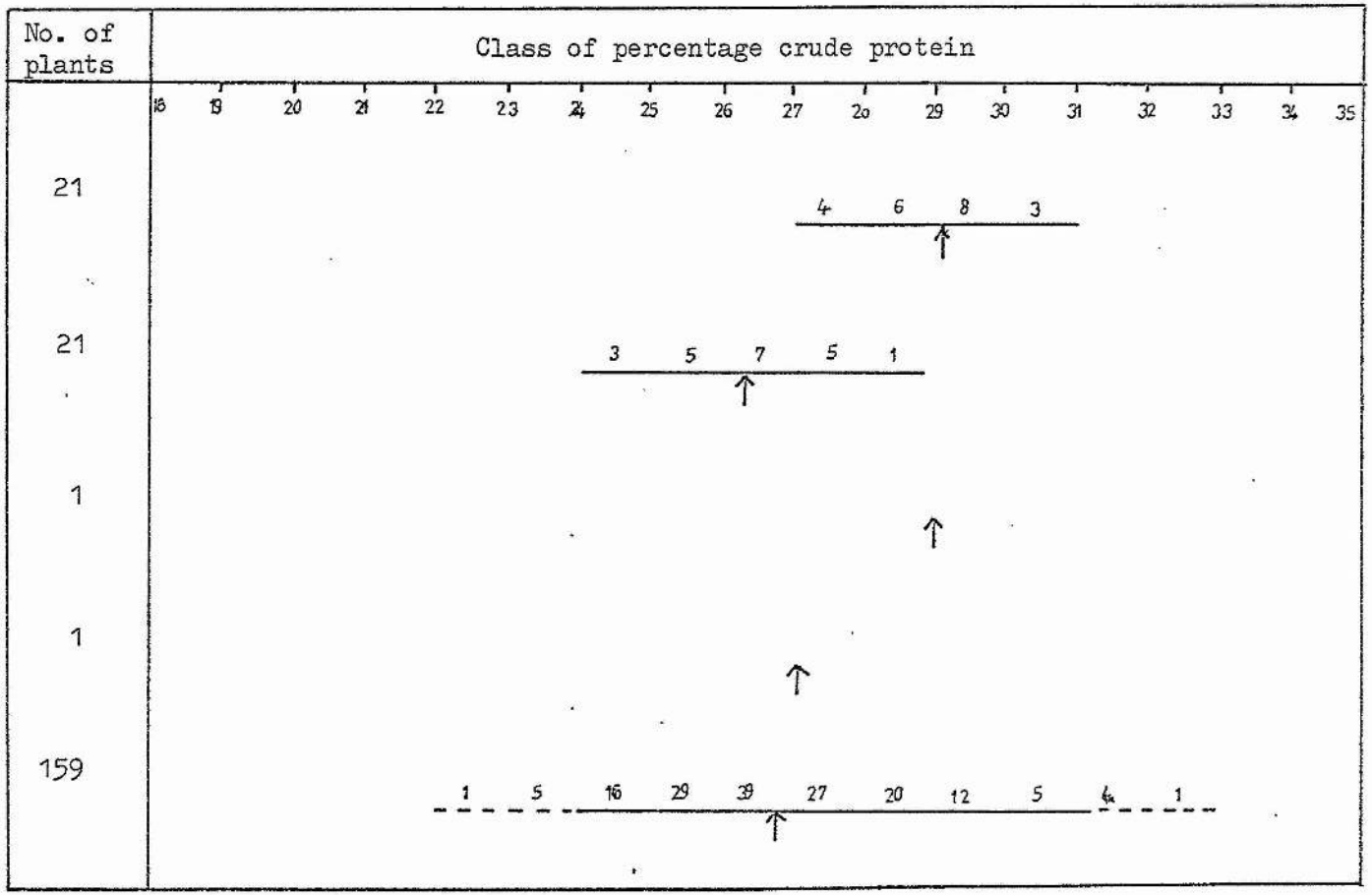
↑ indicates the mean

Table 6.3.13 Frequency distribution of percentage crude protein for the generations of cross 10, Sanilac x W.C.1210



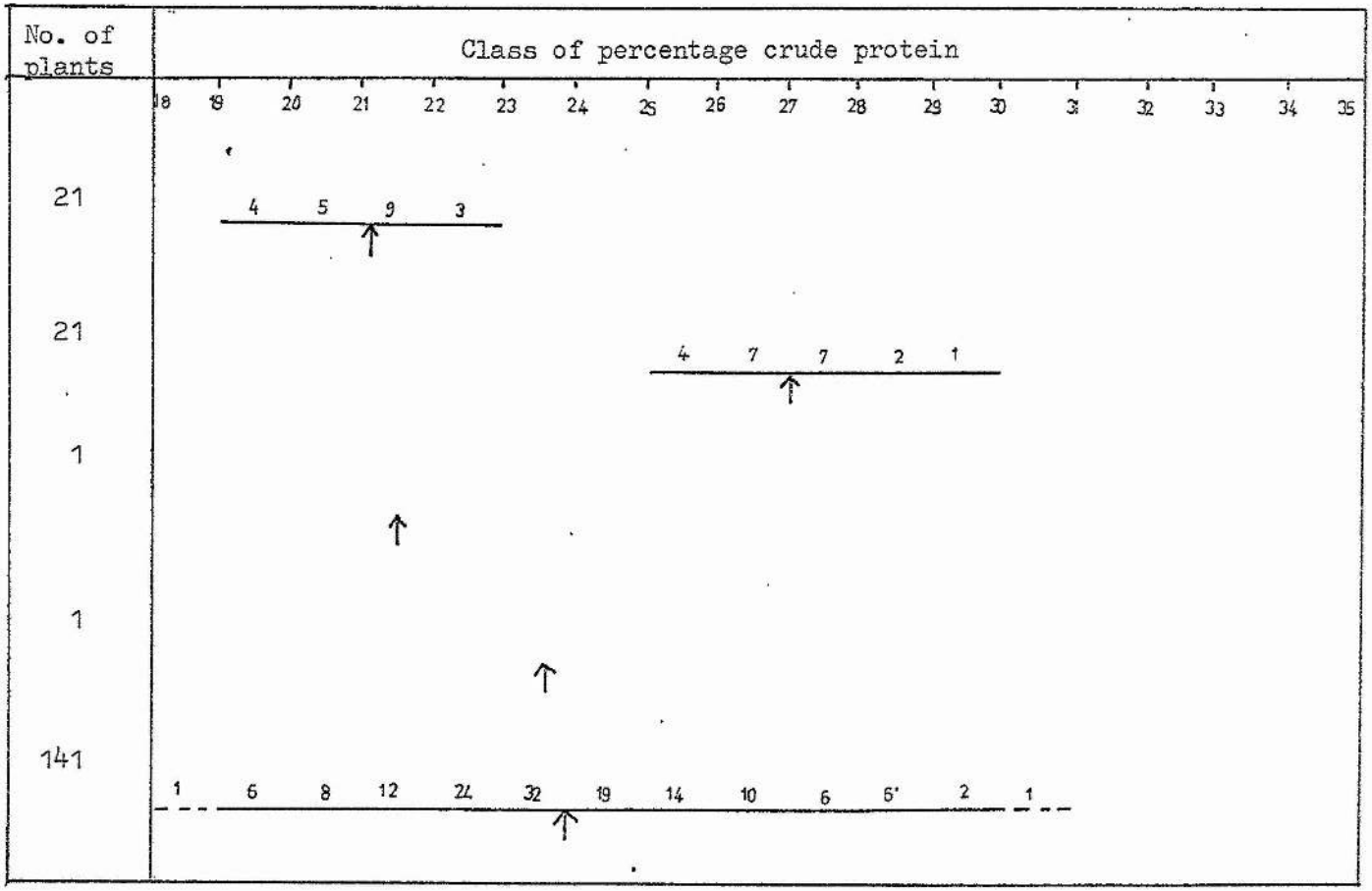
↑ indicates the mean

Table 6.3.14 Frequency distribution of percent crude protein for the generations of cross 11, Sanilac x NEP 2



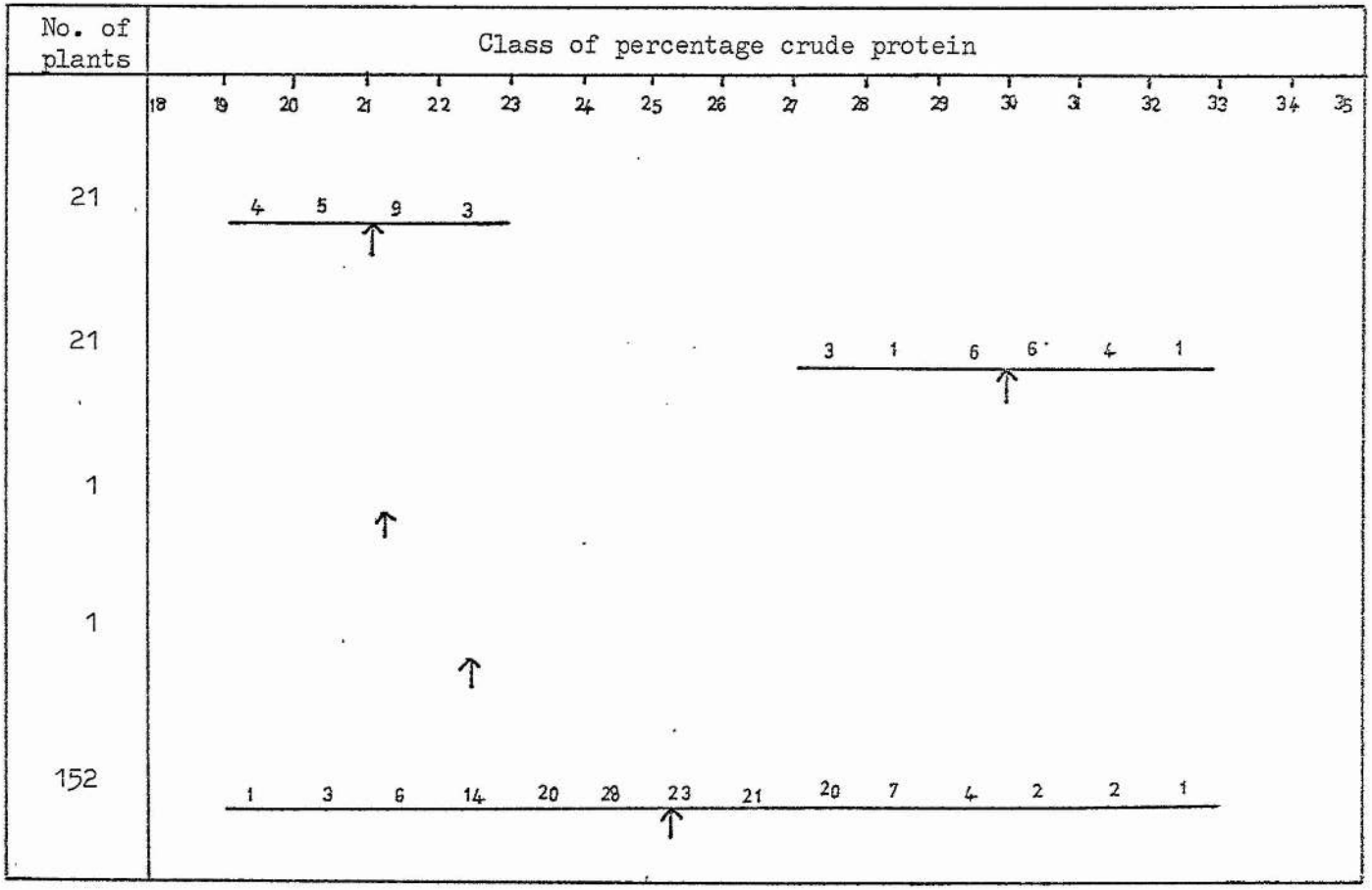
↑indicates the mean

Table 6.3.15 Frequency distribution of percent crude protein for the generations of cross 12, F.M.52 x Kabacuara



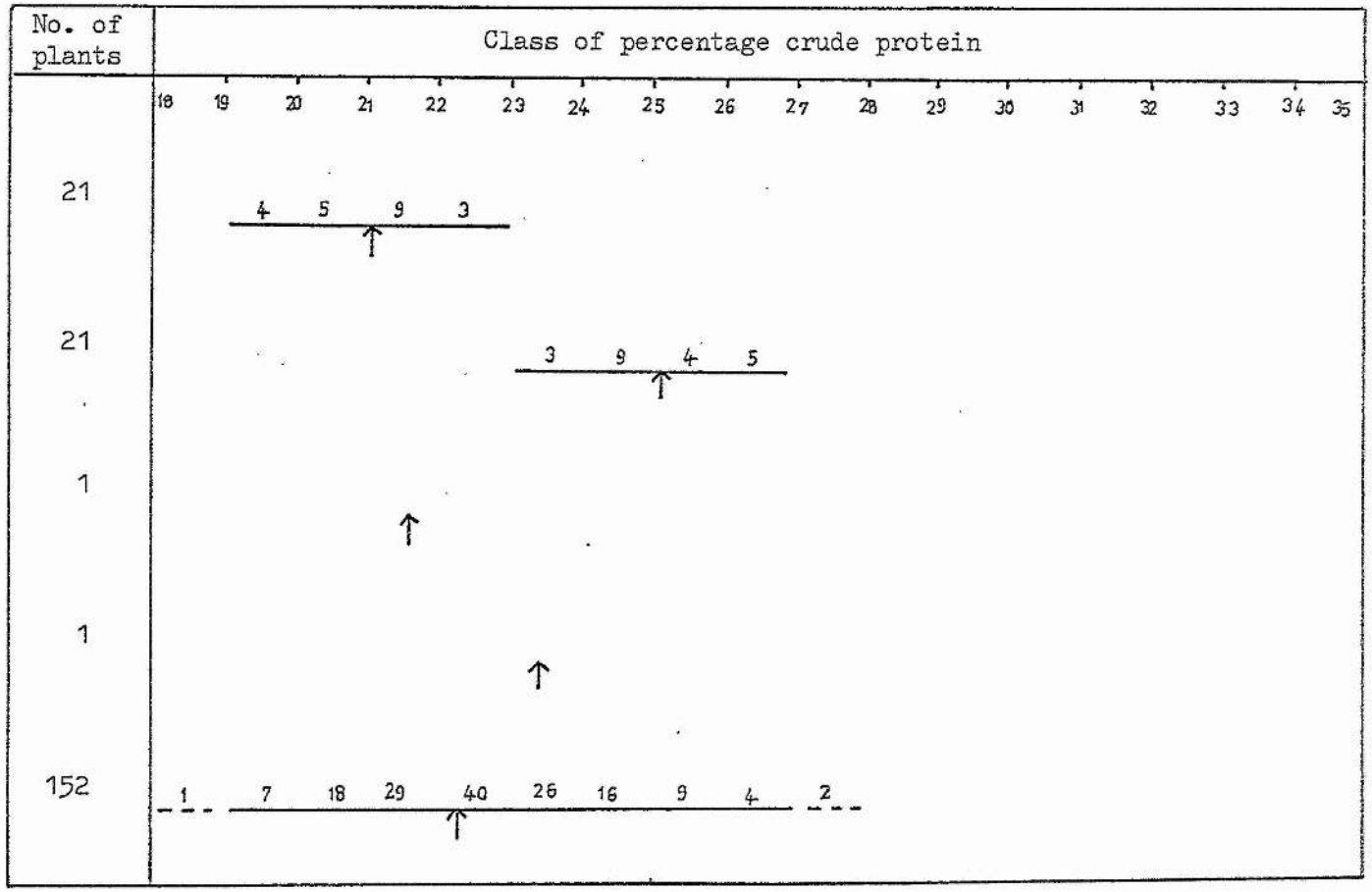
↑indicates the mean

Table 6.3.16 Frequency distribution of percent crude protein for the generations of cross 13, F.M.52 x Pop 412



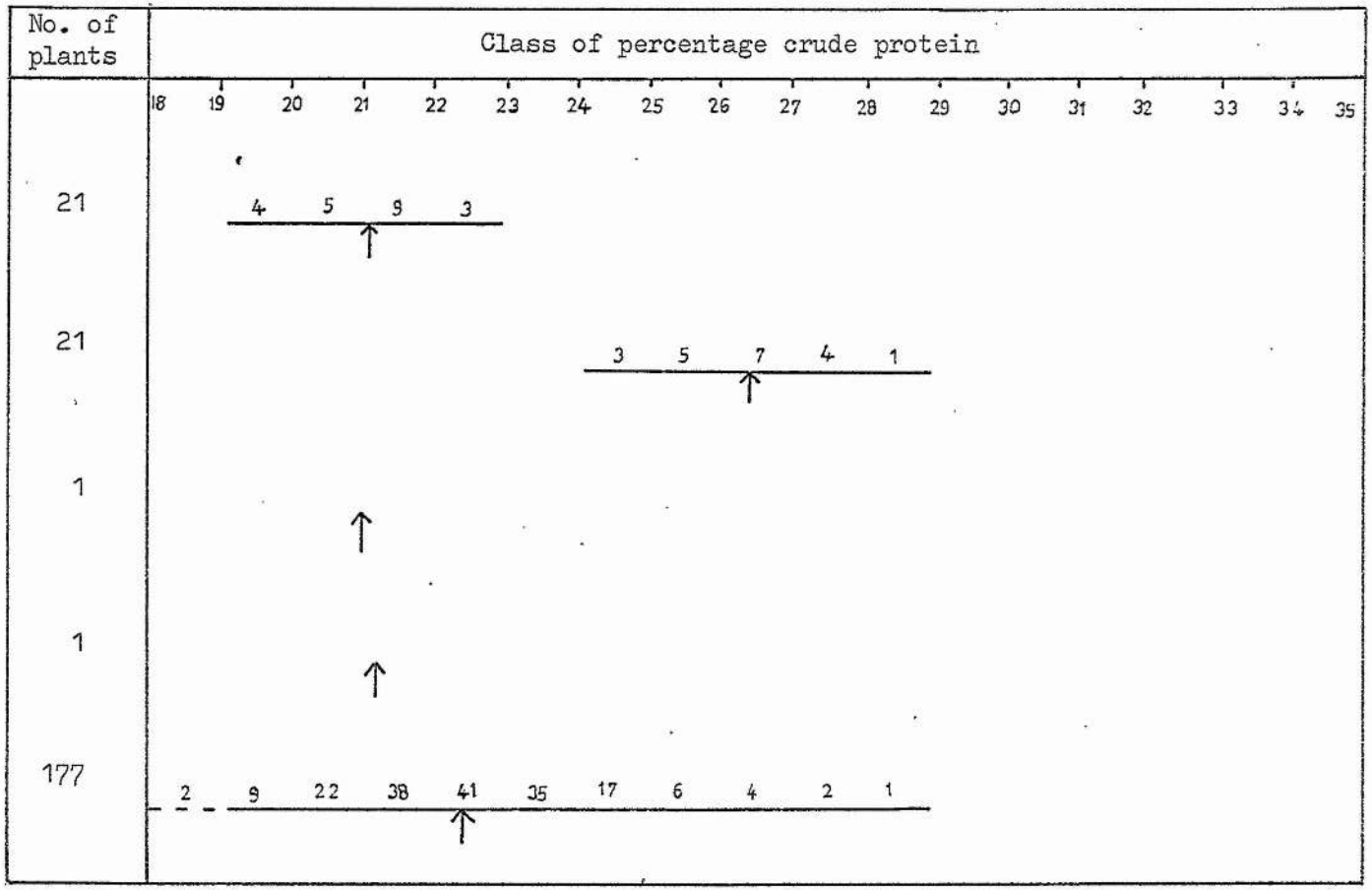
↑ indicates the mean

Table 6.3.17 Frequency distribution of percent crude protein for the generations of cross 14, F.M.52 x W.C.1210



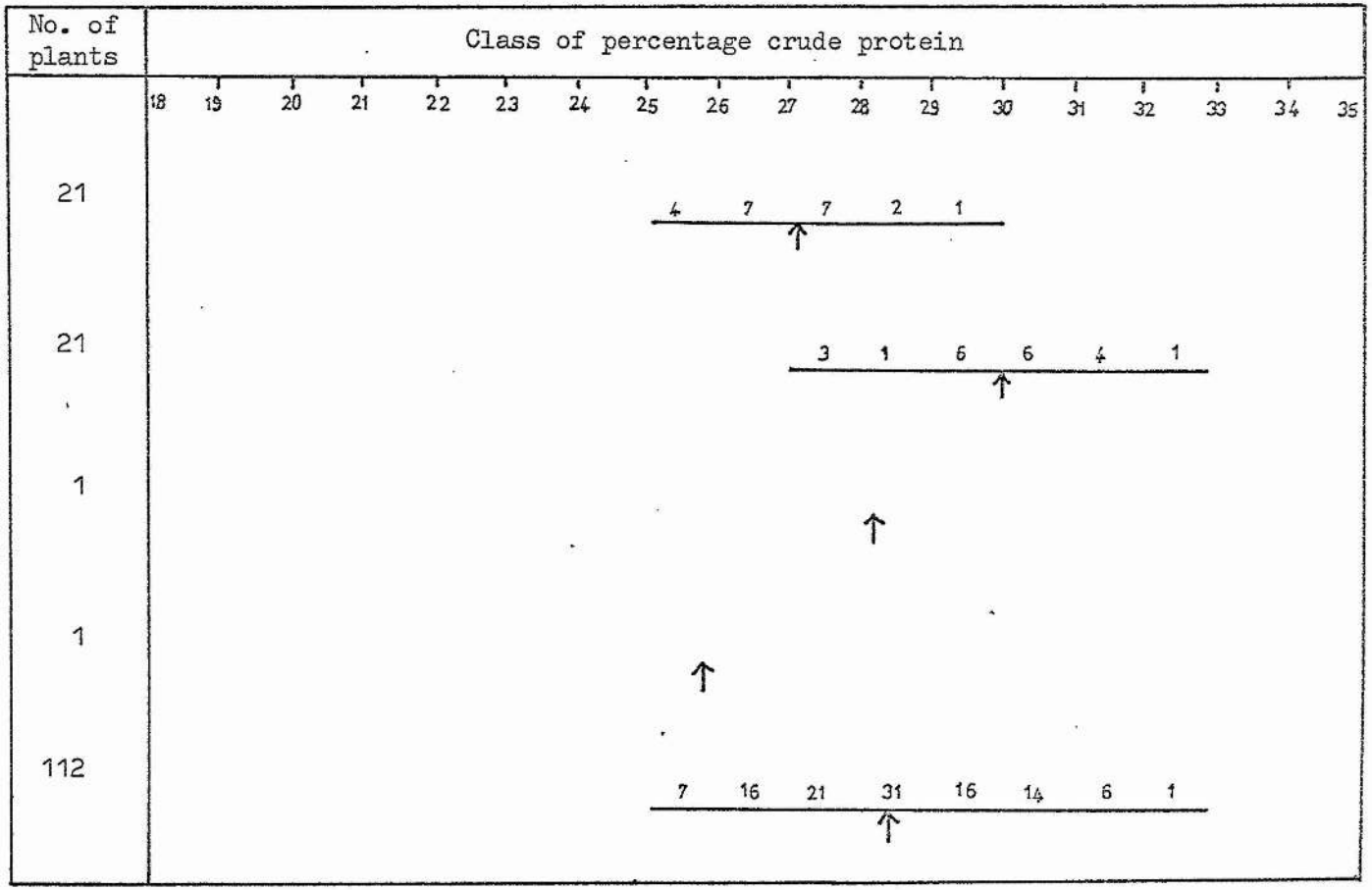
↑ indicates the mean

Table 6.3.18 Frequency distribution of percent crude protein for the generations of cross 15, F.M.52 x NEP2



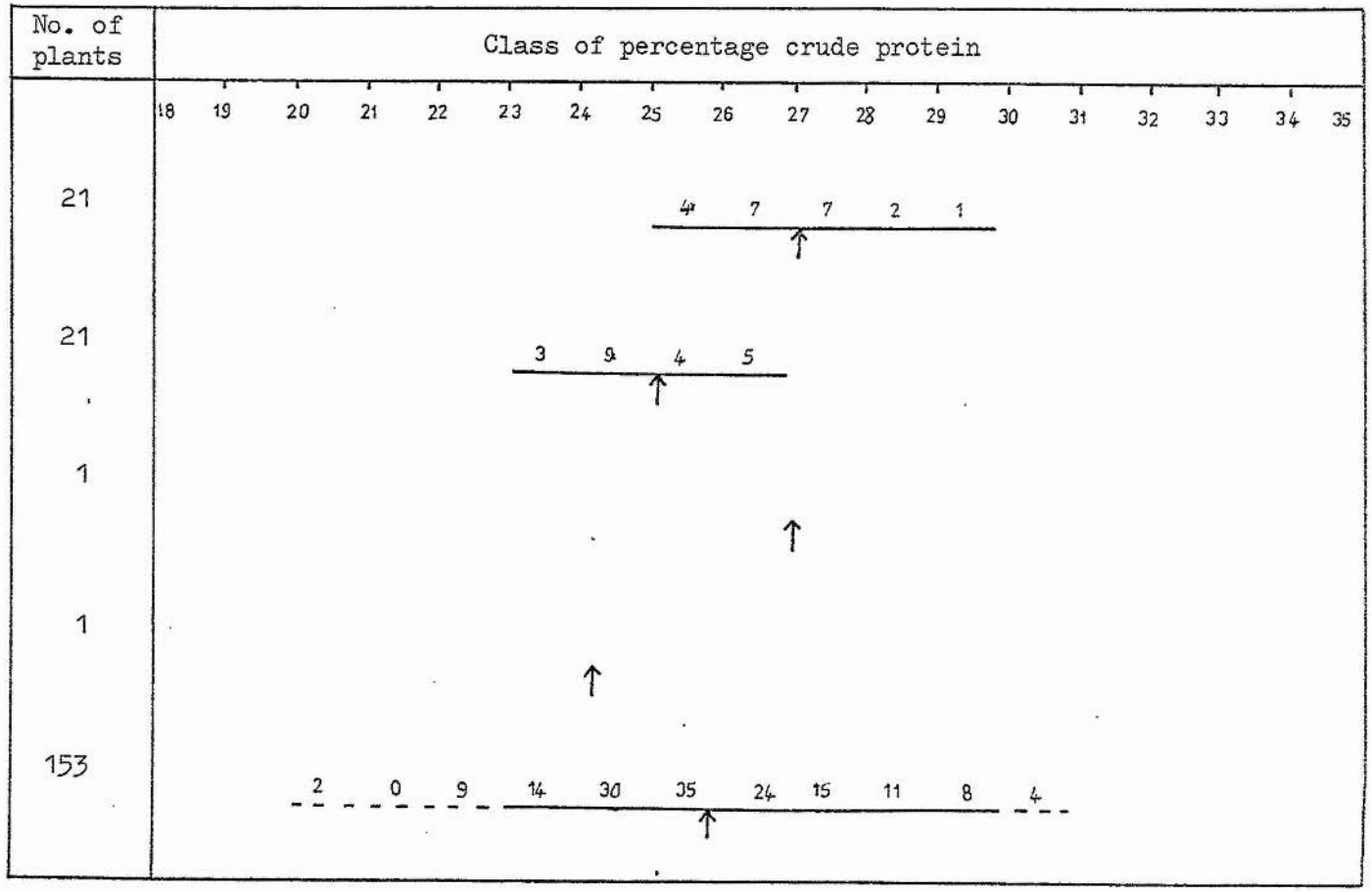
↑ indicates the mean

Table 6.3.19 Frequency distribution of percent crude protein for the generations of cross 16, Kabacuara x Pop 412



↑indicates the mean

Table 6.3.20 Frequency distribution of percent crude protein for the generations of cross 17, Kabacuara x W.C. 1210



↑indicates the mean

Table 6.3.21 Frequency distribution of percent crude protein for the generations of cross 18, Kabacuara x NEP 2

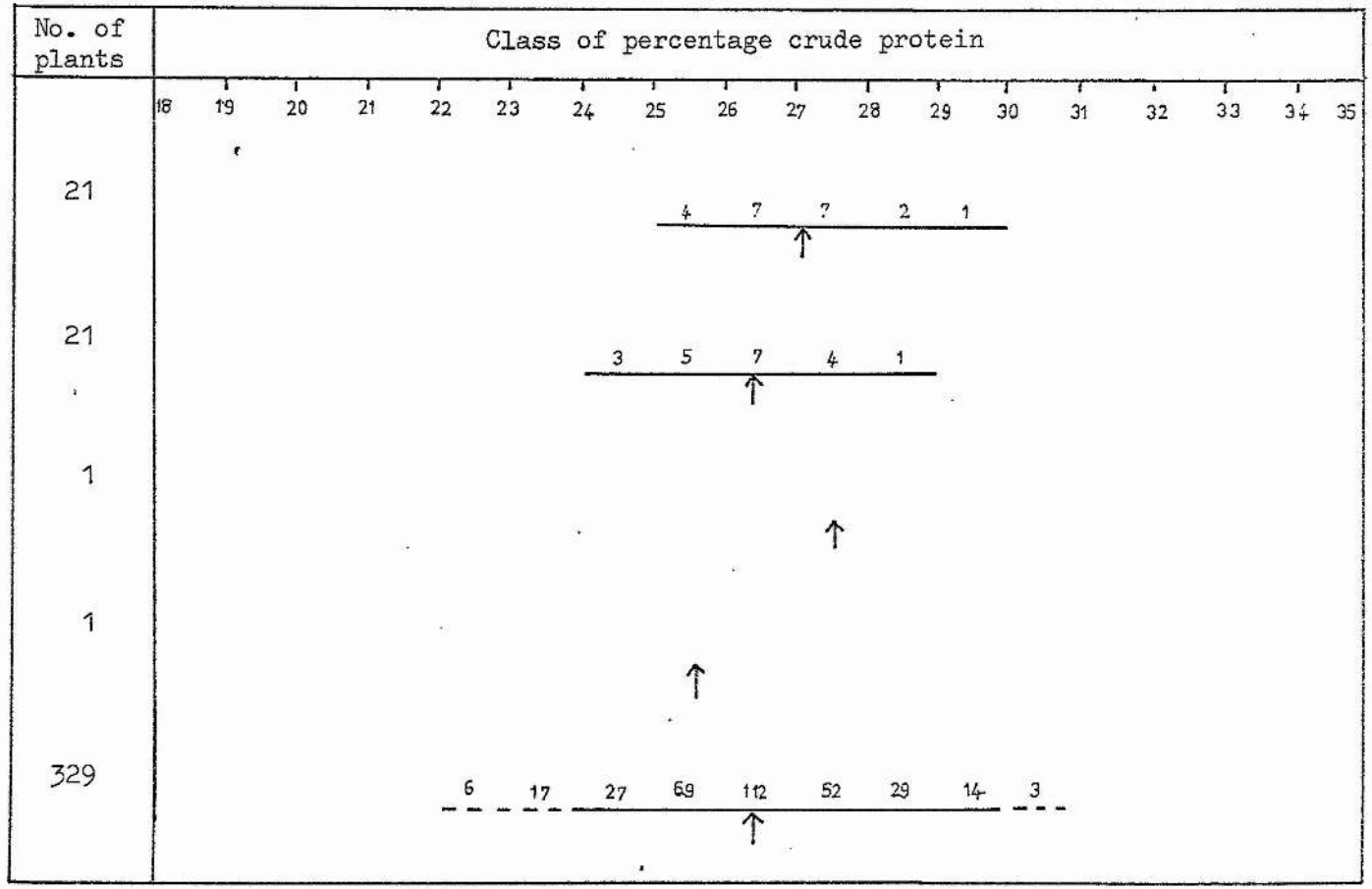
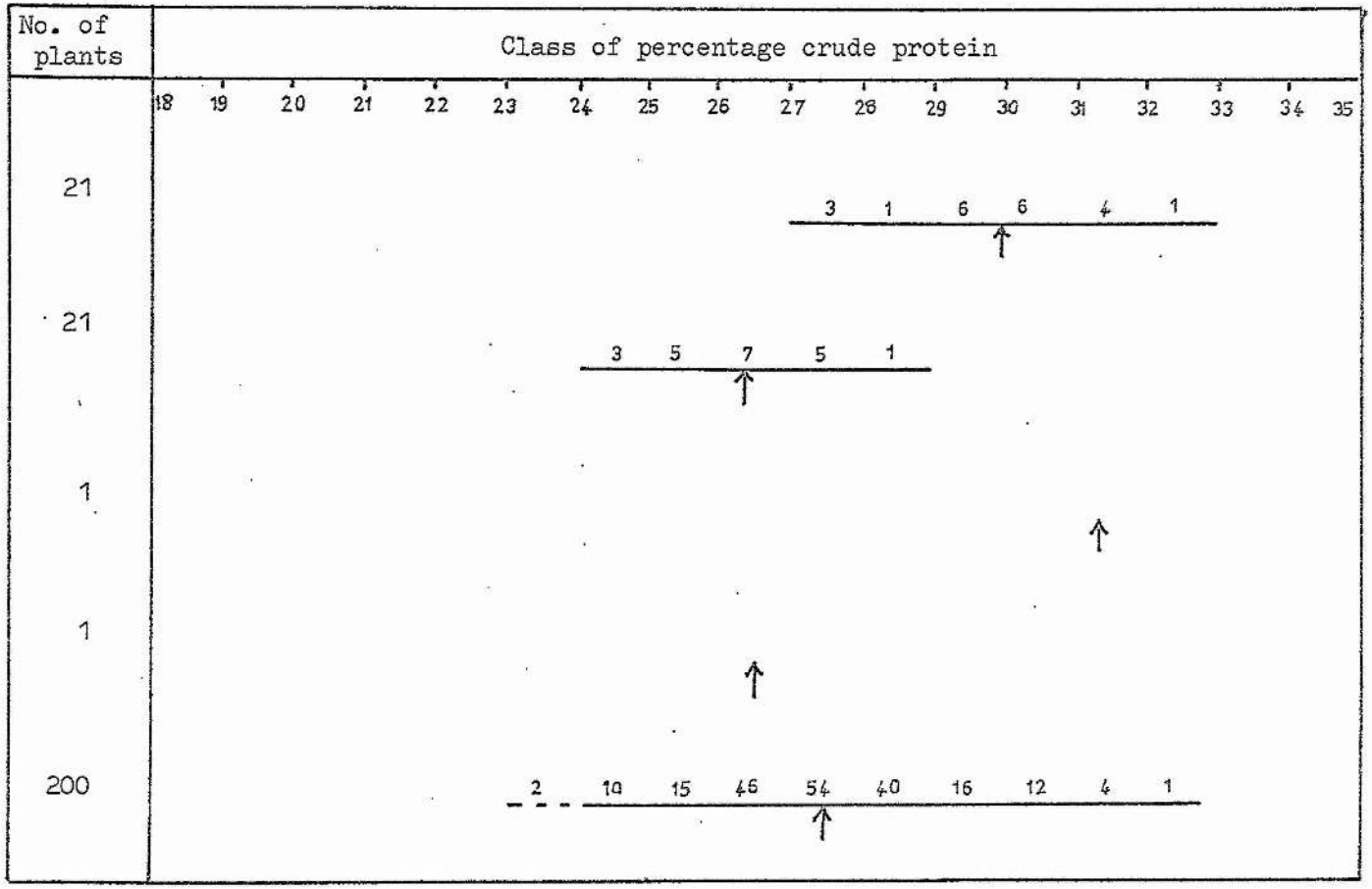


Table 6.3.22 Frequency distribution of percent crude protein for the generations of cross 19, Pop 412 x W.C.1210

	No. of plants	Class of percentage crude protein (18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35)
♀ parent, Pop 412	21	3 1 6 6 4 1
♂ parent, W.C. 1210	21	3 9 4 5
Fo hybrid seed on maternal plant	1	↑
F_1 hybrid plant	1	↑
F_2 generation plants	204	1 6 19 38 56 31 20 14 8 6 4 1

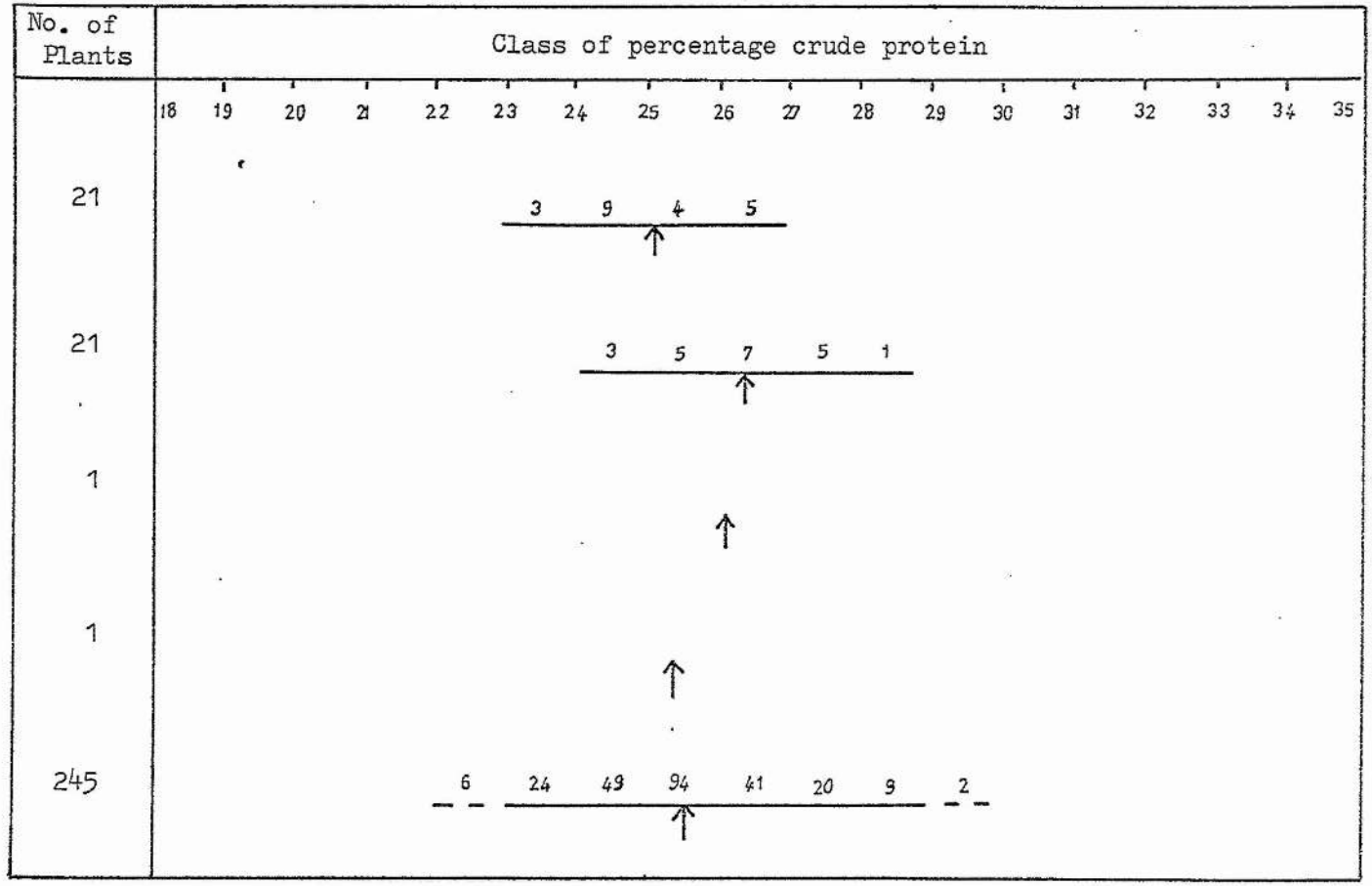
↑ indicates the mean

Table 6.3.23 Frequency distribution of percent crude protein for the generations of cross 20, Pop 412 x NEP 2



↑ indicates the mean

Table 6.3.24 Frequency distribution of percent crude protein for the generations of cross 21, W.C. 1210 x NEP 2.



↑ indicates the mean

Visual inspection of the results showed that:-

Result (1), the protein content of the Fo hybrid single seed was close to the maternal parent. This interpretation was tested for by use of the chi-square analysis for the comparisons (a), (b) and (c) as shown below.

(a) to test if the protein content of the Fo single seed was significantly different from the protein content of the female parent.

(b) to test if the protein content of the Fo single seed was significantly different from the protein content of the male parent.

(c) to test if the protein content of the Fo single seed was significantly different from the protein content of the mid parent.

The analyses showed that the protein content of the Fo single seed was close to the protein content of the female parent, indicating a strong maternal effect.

Result (2), the protein content of the F_1 hybrid plant was close to the low protein parent. For confirmation, chi-square analyses were carried out for three comparisons (a) (b) and (c) as shown below.

(a) to test if the protein content of the F_1 hybrid plant was significantly different from the low protein parent.

(b) to test if the protein content of the F_1 hybrid plant was significantly different from the high protein parent.

(c) to test if the protein content of the F_1 hybrid plant was significantly different from the protein content of the mid parent.

The analyses showed that the protein content of the F_1 hybrid plant was close to the low protein parent and the mid parent and away from the high protein parent. This interpretation was tested for by use of the chi-square analysis for the comparisons (a), (b) and (c) as shown below.

(a) to test if the protein contents of the F_1 hybrids were significantly different from the low protein parents.

(b) to test if the protein contents of the F_1 hybrids were significantly different from the high protein parents.

(c) to test if the protein contents of the F_1 hybrids were significantly different from the female parents.

The analyses showed that the protein contents of the F_1 hybrids were close to the low protein parents and significantly different from the high protein parents. Also the protein contents of the F_1 hybrids were significantly different from the protein contents of the female parents indicating no maternal effects.

Result (3), the protein content results of the F_2 generation plants were normally distributed for all crosses with the exception of crosses 6 and 7 where the distributions are skwede.

In all crosses the mean protein content of the F_2 generation plants was towards the low protein parent.

Result (4), similarly, the protein content of the plants of the F_3 generation were normally distributed with the mean towards the low protein parent.

There was no clear evidence for transgressive segregation for percent crude protein in the genotypes studied. In several crosses, a few plants exceeded the range of either parent. This could generally be explained on the basis of an interaction of yield and protein or as a result of a sampling error. For example, in cross 1, Mexico 142 x Sanilac shows that the average yield of the F_2 generation plants was 14.0 g./plant, and the average protein content 24.6 per cent. The range of protein content of the parents was 23.0 - 31.0 per cent. Several plants exceeded the range. For example, one plant had a measured protein content of 34.1 per cent but its yield was only 6.3g., compared to the mean yield of 14.0 g./plant. This low yield therefore, probably influenced percent crude protein.

The square tables of results from the diallel cross are shown in Tables 6.3.25 - 6.3.27. The results in Table 6.3.25 however are entirely invalidated for the purpose of the diallel analysis since the evidence of maternal effects was overwhelming. However, there was no evidence of maternal effects in future generations. (see page 182). It was therefore assumed that reciprocal differences were absent. Several analyses of variance of the results were first carried out to test the significance of some of the genetical components of variation and also the validity of some of the initial assumptions of the model chosen.

Since reciprocal differences were assumed absent, the analysis of Jones (1965) for the half diallel table was chosen. The determination of the sums of squares was carried out for:-

(a) additive effects

(b1) mean dominance (on the assumption of no epistasis)

(b2) additional dominance effects that can be accounted for by genes having one allele present in only one line (the remaining (n-I) lines being assumed to carry the same alternative allele.)

(b3) residual dominance effects.

The analyses of the 7 x 7 half-diallels for the F_1 hybrid plants and the F_2 generation plants are shown in Tables 6.3.28 and 6.3.29 respectively.

Table 6.3.25 Diallel table showing the mean percent crude protein results for the parents and F_o hybrid seed borne on the maternal plant

	Mexico 142	Sanilac	F.M.52	Kabacuara	Pop412	W.C.1210	NEP 2
Mexico 142	24.6	25.2	24.3	25.2	24.6	25.7	24.2
Sanilac		29.4	28.6	29.8	29.1	29.6	29.0
F.M.52			21.1	21.5	21.3	21.6	21.0
Kabacuara				27.1	28.2	27.0	27.6
Pop412					30.0	26.6	31.4
W.C.1210						25.1	26.1
NEP 2							26.4

Table 6.3.26 Diallel table showing the mean percent crude protein results for the parents and the seeds borne on the F_1 hybrid plants

	Mexico 142	Sanilac	F.M.52	Kabacuara	Pop412	W.C.1210	NEP 2
Mexico 142	24.6	26.6	20.6	26.3	25.8	24.8	25.2
Sanilac	(26.6)	29.4	24.8	28.0	29.8	26.4	27.6
F.M.52	(20.6)	(24.8)	21.1	23.6	22.5	23.4	21.2
Kabacuara	(26.3)	(28.0)	(23.6)	27.1	25.8	24.2	25.6
Pop 412	(25.8)	(29.8)	(22.5)	(25.8)	30.0	25.7	26.5
W.C.1210	(24.8)	(26.4)	(23.4)	(24.2)	25.7	25.1	24.4
NEP 2	(25.2)	(27.6)	(21.2)	(25.6)	26.5	(24.4)	26.4
Array totals (including selfs)	173.9	192.6	157.2	180.6	186.1	174.0	176.9
Array means (including selfs)	24.8	27.5	22.5	25.8	26.6	24.9	25.3

Table 6.3.27 Diallel table showing the mean percent crude protein results for the parents and the F_2 generation plants.

	Mexico 142	Sanilac	F.M.52	Kabacuara	Pop412	W.C.1210	NEP 2
Mexico 142	24.6	25.6	24.7	25.6	28.3	24.6	26.3
Sanilac	(25.6)	29.4	24.1	27.6	28.2	27.5	26.8
F.M.52	(24.7)	(24.1)	21.1	23.9	25.3	22.3	22.4
Kabacuara	(25.6)	(27.6)	(23.9)	27.1	28.4	25.9	26.4
Pop412	(28.3)	(28.2)	(25.3)	(28.4)	30.0	27.0	27.5
W.C.1210	(24.6)	(27.5)	(22.3)	(25.9)	(27.0)	25.1	25.5
NEP 2	(26.3)	(26.8)	(22.4)	(26.4)	(27.5)	(25.5)	26.4
Array totals (including selfs)	179.7	189.2	163.8	184.9	194.7	177.9	181.3
Array means (including selfs)	25.7	27.0	23.4	26.4	27.8	25.4	25.9

Table 6.3.28 Analysis of Variance of the 7 x 7 half-diallel table for the F_1 hybrid plants

Source	df	S.S.	M.S.	F	
a	6	134.38	22.4	20.55	**
b_1	1	5.66	5.66	5.19	*
b_2	6	4.67	0.78	0.72	n.s.
b_3	14	11.85	0.85	0.78	n.s.
Total	27	156.56			
Error$^{\phi}$	120	130.36	1.09		

Table 6.3.29 Analysis of Variance of the 7 x 7 half-diallel table for the F_2 generation plants

Source	df	S.S.	M.S.	F	
a	6	108.84	18.14	16.46	**
b_1	1	0.617	0.617	0.57	n.s.
b_2	6	8.662	1.44	1.32	n.s.
b_3	14	61.71	4.41	4.05	**
Total	27	179.83			
Error$^{\phi}$	120	130.36	1.09		

$^{\phi}$as determined from the replicated protein results of the parents

(see page 156)

** indicates significance at the 1% level

* indicates significance at the 5% level

n.s. indicates non significance

The results indicate significant additive effects with some dominance effects. Thus, protein content can be postulated to be controlled by a series of genes with minor effects which were additive and which showed some dominance relationship.

The assumptions of the diallel model chosen were tested as follows.

(a) parental homozygosity : the parental lines were maintained by controlled self fertilisation in a greenhouse to make sure that there was no apparent genetic variation due to accidental intercrossing. The parents were assumed to be homozygous.

(b) normal diploid segregation : Phaseolus vulgaris has 22 chromosomes (x = 11). It is a diploid and segregates in a diploid manner (Darlington and Ammal, 1945).

(c) no reciprocal differences : this assumption was presumed to be true though later it was found that there was evidence of strong maternal effects on the Fo single seed on the maternal plant. However the results later indicated no strong maternal effects on the F_1 plant and future generations. (see page 182).

The assumptions of (d) no multiple alleles (e) no linkage (f) no interallelic interaction, were tested for together; the procedures were as follows.

(1) analysis of variance of the quantity (Wr-Vr)

(2) analysis of the (Vr,Wr) regression Vr is the variance of the members of an array; Wr is the covariance of the members of an array on the respective parent.

In the first test of the assumptions, the quantity (Wr-Vr) is expected to be constant over arrays if all

assumptions of the analyses are fulfilled (Hayman, 1963; Jinks and Hayman, 1953). Heterogeneity of this quantity over arrays indicates that one or more of the hypotheses are not valid for that particular character. The quantity was calculated for each of the seven arrays in each of the analyses for the F_1 hybrid plant and F_2 generation. The variance values obtained were 0.26 and 0.35 respectively indicating little variation over arrays for the (Wr-Vr) values. Therefore, this test indicates that the assumptions of the analysis are valid.

In the second test, the (Vr,Wr) regression coefficient is expected to be significantly different from 0 but not significantly different from 1.0 if all the assumptions hold true (Jinks & Hayman, 1953). The Vr, Wr regression coefficient for the F_1 hybrid plant is 0.95 and was found to be significantly different from zero but not significantly different from 1. The Vr, Wr regression coefficient for the F_2 generation plants is 1.7 which again was found to be significantly different from zero but not significantly different from 1. Therefore, according to this last test the F_1 hybrid plant and F_2 generation plants results fulfil all assumptions of the diallel.

Thus, according to the tests the results fulfil the assumptions of the diallel and the analysis can therefore be continued.

6.4 Analysis of the data

6.41 Diallel analysis

Data was analysed according to the method of Jinks and Hayman (1953). The parental varieties and progenies were arranged into arrays (see Tables 6.3.26 and 6.3.27). Each array consisted of the parental variety and all the hybrid populations derived from the crosses involving it and their mean values of the percentage crude protein content were used in the analysis (see Tables 6.3.26 and 6.3.27). The variance between parents and within each of the arrays was calculated. The covariance of each array with the common parent was also calculated. This being the covariance of the parents with their respective offspring making up a particular array. Regression analysis of array covariances as the dependent variables on the variances of the arrays was carried out. The results are presented in a graphical form with the covariance of the array, Wr as the ordinate and the variance of the array, Vr as the abscissa with the calculated linear regression line from the analysis fitted. The positions of the arrays are described by their values of variance and covariance within the limiting parabola described by the equation $(Wr)^2 = Vr\ V_{\bar{p}}$ where $V_{\bar{p}}$ is the variance of the parental means. The line of regression in the form $Wr = a + b\ Vr$ cuts the Wr axis at the point 'a' where $a = Wr - bVr$. Where b is the coefficient of regression.

Interpretation of the genetic properties of the parental varieties has been based on visual examination of the graphical representation of the results of the diallel cross analysis and the fitted regression line. Two features of these graphs were particularly noted. They were:-

(1) the slope and position of the regression line upon which points representing the parental arrays fall and

(2) the scatter of the points along this regression line.

The position of the line shifts with changes in dominance. With no dominance, the line becomes a tangent to the parabola and there is full additivity. With full dominance at all loci the line passes through the origin. Overdominance shifts the line down and with partial dominance the line is shifted up i.e. it lies between the position of no dominance (full additivity) and full dominance. A graphical representation of the degrees of dominance is shown in Fig. 6.41.1 as described by Jinks (1954)

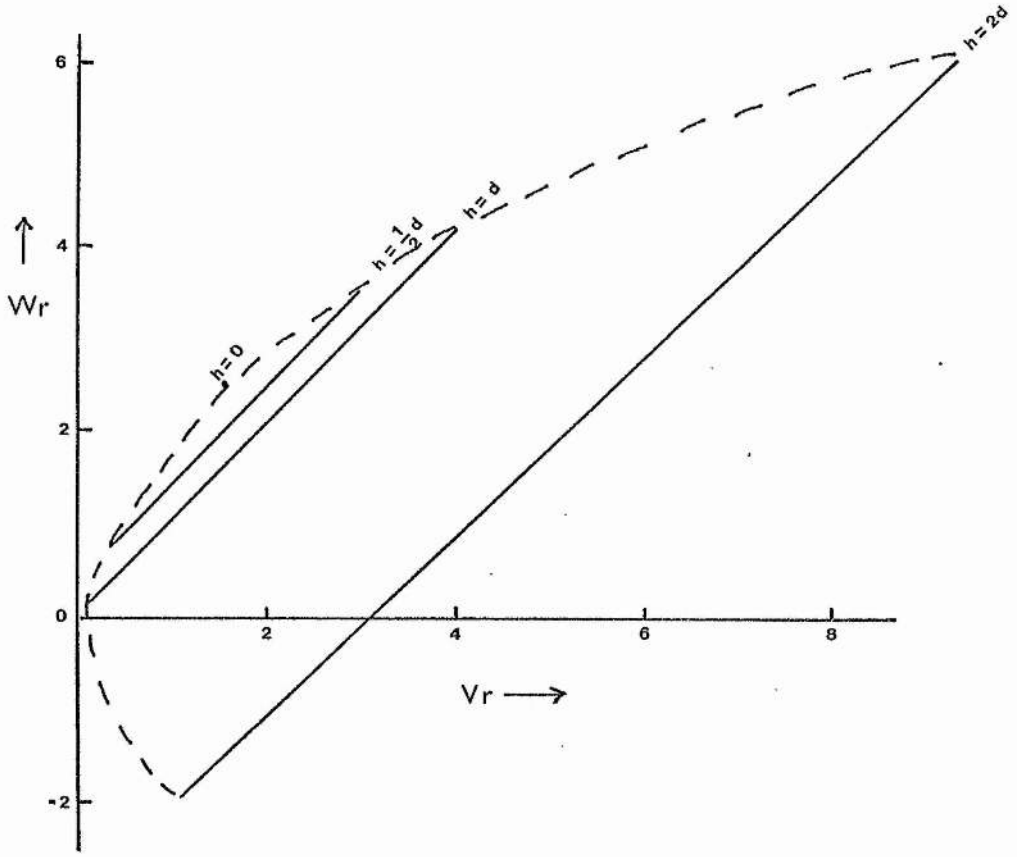


Fig. 6.41.1 The theoretical regressions of Wr on Vr for various degrees of dominance. The curve (broken line) joins the points of the arrays whose common parents contain all the dominant or all the recessive alleles.

When the genes differ in their effectiveness and in their degree of dominance the position of the fitted regression line gives a weighted estimate of their mean effectiveness. If the heterogeneity of the level of dominance between varieties is great, then a significant linear regression will not be obtained. Position on the regression line depicts the order of the number of dominant genes in the parents whether these be for positive or negative expression of the trait and the spread of the points along the line provides a measure of the genetical diversity of the parents. If dominance were to be full and undirectional at all loci (a most unlikely possibility) the top dominant parent lies at the origin and the bottom recessive at the intersection of the regression line and the limiting parabola. In the absence of the top dominant and bottom recessive parents, the points representing the most dominant and most recessive parents present, occupy positions inside the limiting parabola at distances reflecting the number and effectiveness of the dominant and recessive alleles of the extreme genotypes.
The graphs for each of the diallel analyses are shown in Figs. 6.41.2 and 6.41.3. The varieties or their arrays were denoted in the graphs as follows:-

MEX = Mexico 142

SAN = Sanilac

FM = F.M. 52

KAB = Kabacuara

POP = Pop 412

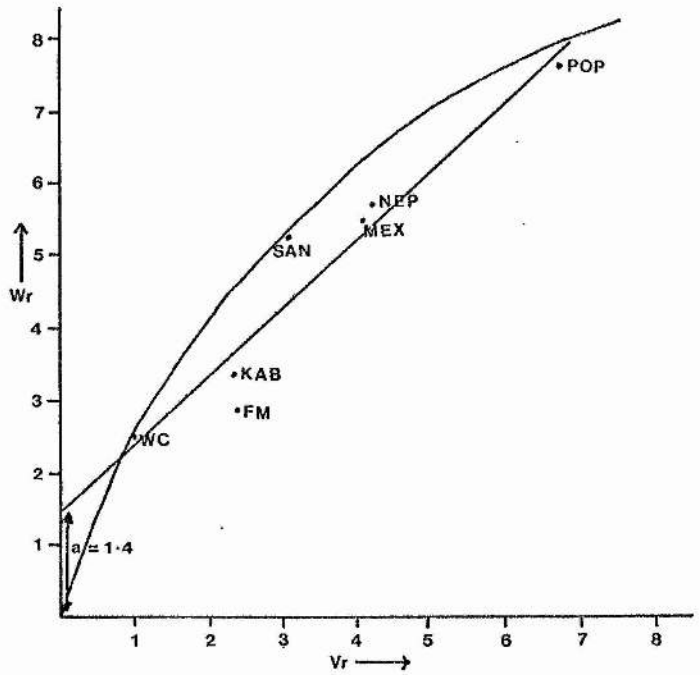
WC = W.C. 1210

NEP = NEP 2

Fig. 6.41.2 Wr, Vr graph for the seeds borne on the F_1 hybrid plant

Array	Vr	Wr
MEX	4.1	5.5
SAN	3.1	5.3
F M	2.4	2.9
KAB	2.4	3.4
POP	6.8	7.6
W C	1.0	2.5
NEP	4.3	5.7

$\bar{V}_p = 9.2$



Regression coefficient = 0.95 ± 0.04

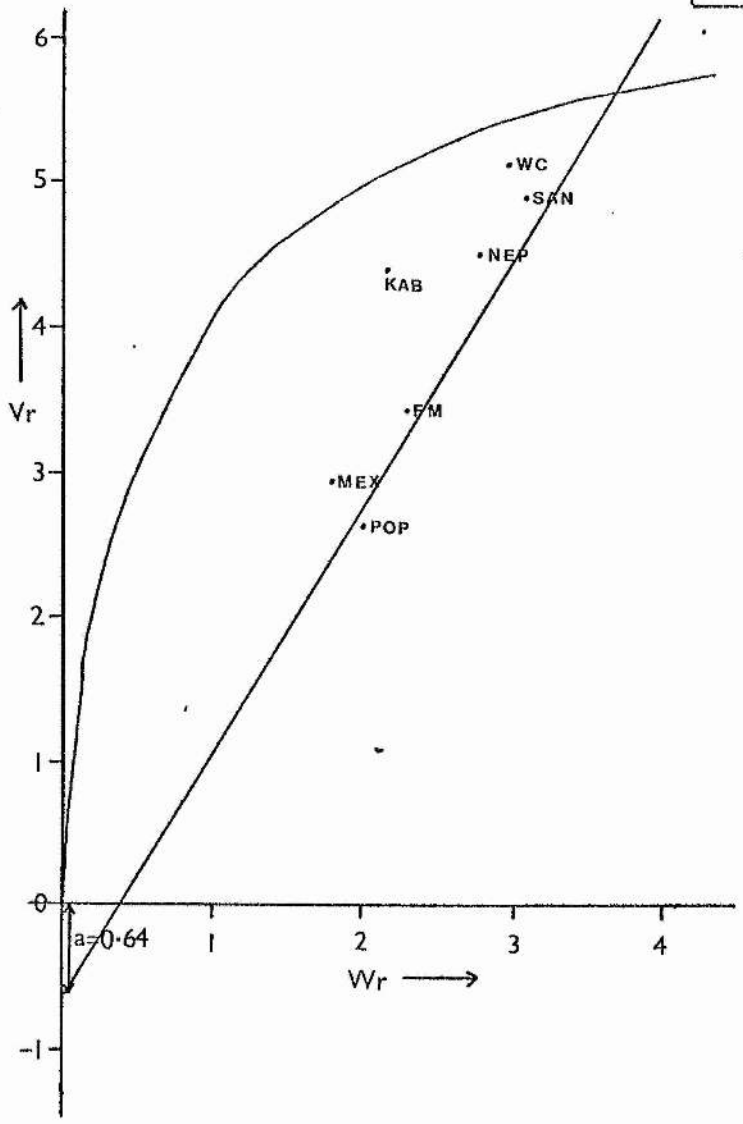
't' = 21.9 P = 0.1%

∴ a significant relationship between Vr and Wr.

Fig. 6.41.3 Wr, Vr graph for the seeds on the F_2 generation plants

Array	Vr	Wr
MEX	1.8	2.9
SAN	3.1	4.9
FM	2.3	3.4
KAB	2.2	4.4
POP	2.0	2.6
W C	3.0	5.1
NEP	2.8	4.5

$\bar{V}_p = 9.2$



Regression coefficient, b = 1.7 ± 0.77

't' = 2.77 P = 5%

∴ a significant relationship

between Vr and Wr

Discussion of the diallel analysis

Fig. 6.41.2 shows the values of the variances and covariances of the arrays of the F_1 hybrid plants, together with a graph showing the linear regression (Vr, Wr) and the parabola, $(Wr)^2 = V\bar{p}\ Vr$. This graph can be interpreted as follows : the parabola marks the limits within which the points Vr, Wr should lie. Since the regression of (Vr, Wr) is 0.95, it agrees with a slope of 1. The gene system can therefore be deduced to be additive without the complications of gene interaction. The position of this line being near to the parabola indicates some dominance. The order of the array points on the line indicates the distribution of dominant and recessive genes amongst the common parents of the arrays, WC possessing the most dominants and Pop the most recessives.

Fig. 6.41.3 shows a graphical analysis of the F_2 generation data for protein. The position of the regression line indicates average overdominance in all arrays. The regression coefficient is significantly different from zero but not from the value of one. The gene system can therefore be deduced again to be additive without complications of gene interaction. The order of the array points on the line indicates a distribution of dominant and recessive genes amongst the common parents of the arrays which is different to that of the F_1 generation. The results therefore may question the adequacy of the Hayman Jinks model and analysis for the results of the present study. However, the low protein array FM and the high protein array SAN have remained in approximately the same position on the regression line for both analyses - the low protein array being in the dominant genes position and the high protein array being in the recessive genes position. This interpretation of the results together with the protein results of the parents, Fo, F_1, F_2 and F_3 generations (see Tables 6.3.4 -

6.3.24) and the analysis of variance of the genetic components of variation can be assumed to deduce that protein content is controlled by a series of genes with minor effects which are additive and which show a dominance relationship for low protein in some of the loci.

6.42 General and specific combining ability

The estimates for general and specific combining ability of the populations for protein content were calculated for both the F_1 and F_2 generations in the 7 x 7 diallel. The method used was that of Griffing (1956) using his method for parents and one set of a hybrid population, excluding reciprocals (i.e. $\frac{1}{2}p\ (p + 1)$ combinations where $p = 7$). The results for the general combining ability (G.C.A.) and specific combining ability (S.C.A.) for the F_1 and F_2 generations are shown in Tables 6.42.1 and 6.42.2.

The varieties which showed the best overall general combining ability for protein content were Pop 412 and Sanilac.

Table 6.42.1 Estimates of General and Specific Combining Ability for protein content in the F_1 generation

	G.C.A.	S.C.A.						
		Mexico 142	Sanilac	F.M.52	Kabacuara	Pop412	W.C. 1210	NEP 2
Mexico 142	-0.58	X	1.76	-3.32	1.71	0.89	0.43	0.69
Sanilac	2.07		X	-3.9	-1.37	0.11	-2.3	-1.69
F.M.52	-2.82			X	3.12	1.69	2.14	0.79
Kabacuara	0.44				X	-8.8	-1.93	-0.67
Pop 412	1.38					X	-1.97	-1.2
W.C.1210	-0.47						X	-0.27
NEP 2	-0.04							X

Table 6.42.2 Estimates of General and Specific Combining Ability for protein content in the F_2 generation

	G.C.A.	S.C.A.						
		Mexico 142	Sanilac	F.M.52	Kabacuara	Pop412	W.C. 1210	NEP 2
Mexico 142	-0.4	X	-0.13	-0.11	0.13	2.5	-0.65	0.9
Sanilac	1.18		X	-3.35	-0.52	-0.24	-0.4	-1.24
F.M.52	-2.55			X	2.4	3.5	1.0	0.93
Kabacuara	0.45				X	1.23	-0.78	-0.43
Pop 412	1.86					X	-2.18	-1.83
W.C.1210	-0.54						X	0.45
NEP 2	-0.22							X

The analysis of variance of the effects due to general and specific combining abilities for both generations, are shown in Table 6.42.3.

Table 6.42.3 Analyses of Variance for Combining Abilities

F_1	df	SS	MS	F	
G.C.A.	6	125.34	20.89	19.165**	
S.C.A	21	26.12	1.24	1.138	n.s.
Error$^{\phi}$	120	130.36	1.09		

F_2	df	SS	MS	F	
G.C.A.	6	108.8	18.13	16.63**	
S.C.A.	21	11.07	0.53	0.486	n.s.
Error$^{\phi}$	120	130.36	1.09		

$^{\phi}$where the error is derived from the randomised block analysis.

**indicates significance at the 1% level.

n.s. indicates non significance

The results indicate that only significant effects were obtained for the general combining ability for both the F_1 and F_2.

6.43 Heritability estimates

The concept of heritability originated as an attempt to describe whether similarities between parents and their offspring arose from the similarities in their genetic make-up or from similar environmental forces. Knight (1948) defines heritability as "the portion of observed variance between progenies for which difference in heredity is responsible". The basic concept of heritability is simple. Discrepancies in measurement arise depending on how exactly it is indexed and applied to different breeding situations.

Heritability is used in both a broad and a narrow sense. For broad sense, the genotype is considered as the unit in relation to the environment. However, genes segregate and come together in new combinations exhibiting intra-allelic interactions (dominance) and inter-allelic interactions (epistasis). The differences between the actual effects of particular combinations of genes and their average effects in all genotypes containing them in the population result from gene interaction (non additivity). Such interaction may be due to intra-allelic effects (dominance) and inter-allelic effects (epistasis). Heritability in the broad sense considers total genetic variability in relation to the phenotypic variability while heritability in the narrow sense considers only the additive portion of the

genetic variability in relation to the phenotypic variability. Thus, heritability in the narrow sense expresses the function of the phenotypic differences between parents which one expects to recover in the offspring and is designed to give a practical interpretation to heritability. It is narrow sense heritability estimates which are considered to be of most value. They can then be used to estimate expected improvement due to selection.

Both broad and narrow sense heritability estimates were determined by the methods outlined by Mahmud and Kramer (1951). Broad sense heritability estimates were calculated for all families using the formula:

$$H = \left[\frac{VF_2^2 - \sqrt{VP_1^2 \; V_{P_2}^2}}{VF_2^2} \right] X \; 100$$

where V = Variance

H = heritability

$F_2 = F_2$ variance

$P_1 = P_1$ variance

$P_2 = P_2$ variance

Narrow sense heritability estimates were determined from the regression of F_3 plants on F_2 plants. In this case the formula was

$$H = \frac{\bar{\bar{x}} \quad bxy}{\bar{\bar{y}}} \times 100$$

H = heritability

$\bar{\bar{x}}$ = mean of F_2 generation

$\bar{\bar{y}}$ = mean of F_3 generation

bxy = regression coefficient of y on x

The number of plants of the P_1, P_2, F_2, and F_3 generations used for the calculations of both the broad and the narrow sense heritability estimates are listed in Tables 6.3.4 - 6.3.24.

The broad sense heritability estimates are listed in Table 6.43.1; the narrow sense heritability estimates in Table 6.43.2.

Table 6.43.1 Broad sense heritability estimates of crude protein based on parental and F_2 variance for each of the diallel cross families

Cross No.	Cross	Heritability %
1	Mexico 142 x Sanilac	98
2	Mexico 142 x F.M.52	97
3	Mexico 142 x Kabacuara	89
4	Mexico 142 x Pop 412	94
5	Mexico 142 x W.C.1210	96
6	Mexico 142 x NEP 2	91
7	Sanilac x F.M.52	99
8	Sanilac x Kabacuara	82
9	Sanilac x Pop 412	81
10	Sanilac x W.C.1210	93
11	Sanilac x NEP 2	89
12	F.M.52 x Kabacuara	97
13	F.M.52 x Pop 412	94
14	F.M.52 x W.C.1210	89
15	F.M.52 x NEP 2	89
16	Kabacuara x Pop412	75
17	Kabacuara x W.C. 1210	94
18	Kabacuara x NEP 2	81
19	Pop 412 x W.C. 1210	86
20	Pop 412 x NEP 2	66
21	W.C.1210 x NEP 2	60

Table 6.43.2 Narrow sense heritability estimates based on the regression of F_3 plants on F_2 plants for six of the diallel cross families

Cross No.	Cross	Heritability
1	Mexico 142 x Sanilac	24
2	Mexico 142 x F.M.52	8
3	Mexico 142 x Kabacuara	18
4	Mexico 142 x Pop 412	31
5	Mexico 142 x W.C.1210	17
6	Mexico 142 x NEP 2	24

Discussion of the heritability estimates

The broad sense heritability estimates ranged from 60-99 per cent; the narrow sense heritability estimates from 8-31 per cent. As expected, the broad sense heritability estimates were far larger than the narrow sense estimates. This indicates the strong contribution of non-additive genetic variation.

The broad and narrow sense heritability estimates must be treated with some caution since the experiment was carried out under field conditions i.e. no control of the environment. Also caution must be taken when comparing these estimates with those of other workers since different breeders work with different genetic material and under different environmental conditions. Only one estimate of protein heritability of beans Phaseolus vulgaris, is reported in the literature by Lelegi et al (1972). They reported broad sense heritability estimates ranging from 30-64 per cent and narrow sense heritability estimates from 5-20 per cent. They calculated broad sense heritability estimates using the formula given by Burton (1951) i.e. $H = \frac{V_{F2} - V_{F1}}{V_{F2}}$ where V_{F1} and V_{F2} are the variances of the F_1 and F_2 populations of each cross in question. For narrow sense heritability estimates they used the F_2/F_3 regression method of Mahmud and Kramer (1951) and the backcross method of Warner (1952). The estimates of Lelegi et al were similar in range to those of this study also in the fact that the broad sense heritability estimates were far greater than the narrow sense heritability estimates.

Since heritability in the narrow sense expresses the fraction of the range of phenotypic values of the parents that one expects to recover in the offspring under random mating, then for protein in

beans it can be postulated that one would expect a gain of about 5-30 per cent. This information could be valuable for use in planning breeding programmes. The low percent additive gene action indicates that progeny testing would be essential.

CHAPTER 7 RELATIONSHIP BETWEEN PROTEIN CONTENT AND YIELD

7.1 Introduction

An increased protein percentage is desirable in beans, though the first priority is increased yield. Dry beans are an important source of protein but there is very little literature relating to protein content and its correlation with seed yield.

Rutger (1971) reported that yield and protein content were not significantly correlated, indicating the possibility of breeding high yielding, high protein cultivars of beans. Silbernagel (1971) reported that seed yield was negatively correlated with protein percentage in beans while Lelegi et al (1972) reported that there were negative as well as positive correlations between seed yield and percentage crude protein. This chapter therefore propose to consider the protein/yield relationship beans grown under differing conditions.

7.2 Materials and methods

Experimental results for yield and percentage crude protein were taken from:

(1) results from the entire collection of lines of white haricot beans in Uganda (Chapter 4)

(2) results from the 25 selected varieties as grown over four different seasons (Chapter 5)

(3) results from the 4 varieties grown under different levels of nitrogen (Chapter 5)

(4) results from the parents and the large numbers of plants of segregating generations of hybrids (Chapter 6)

7.3 Results

The results are shown in Tables 7.3.1 - 7.3.7.

Table 7.3.1 Results of the correlation of percentage crude protein with seed yield in g. per plant for the entire collection of white haricot beans

n	correlation coefficient	standard error	't'	probability
166	-0.2078	± 0.0745	2.7821	0.01

Table 7.3.2 Results of the correlation of percentage crude protein with seed yield in g. per plant for the 25 varieties grown in the four different seasons

Variety No.	Correlation coefficients*			
	1st Rains 1971	2nd Rains 1971	1st Rains 1972	2nd Rains 1972
V1	-0.1327	0.7966	-0.9319	-0.5670
V2	-0.2552	0.9160	-0.5741	0.0612
V3	0.7897	-0.9965	-0.6025	-0.9143
V4	-0.8306	-0.2181	0.2060	0.6676
V5	0.9951	-0.9051	-0.9489	0.8814
V6	0.1992	-0.2259	0.3312	-0.2692
V7	0.3534	0.4632	0.1324	0.2410
V8	-0.5736	-0.8442	-0.5998	0.7840
V9	0.9664	-0.8698	-0.4658	0.3538
V10	0.9971	-0.9174	0.0675	-0.3216
V11	-0.4859	-0.9776	0.5365	-0.7545
V12	-0.1502	-0.8009	0.5210	-0.3550
V13	-0.3158	0.7600	-0.9971	-0.4695
V14	-0.3942	-0.8230	-0.7411	-0.9858
V15	-0.3554	0.9993	0.5874	-0.8485
V16	-0.9990	-0.5064	0.9563	0.3182
V17	-0.8146	-0.9994	-0.9795	-0.8481
V18	-0.7372	-0.9785	-0.7741	0.2912
V19	-0.8660	-0.6989	-0.5421	-0.3045
V20	0.9707	-0.3338	0.0463	-0.9261
V21	-0.2952	0.8159	-0.6042	-0.7107
V22	-0.9301	0.6781	-0.5169	0.7206
V23	-0.1217	-0.2105	-0.9237	0.9874
V24	-0.8546	-0.7082	0.8762	-0.4555
V25	0.9995	-0.8139	-0.0227	0.8282

*each correlation coefficient is derived from 3X and 3Y values

Table 7.3.3 Results of the correlation of percentage crude protein with seed yield in g. per plant for the varieties grown under different levels of fertiliser

Variety	n	Correlation coefficient	Standard error	't'	Probability
F.M. 53	15	0.0953	± 0.2642	0.3457	0.7
Mexico 142	15	-0.3494	± 0.2341	1.4367	0.2
Sanilac	15	-0.5036	± 0.1990	2.4357	0.02
Kabacuara	15	-0.4532	± 0.2119	2.0590	0.05

Table 7.3.4 Results of the correlation of percentage crude protein with seed yield in g. per plant for the parents of the diallel cross

Variety	n	Correlation coefficient	Standard error	't'	Probability
Mexico 142	21	0.3322	±0.1989	1.6278	0.1
Sanilac	21	-0.2530	±0.1670	1.4763	0.2
F.M.52	21	-0.3192	±0.1522	2.0438	0.05
Kabacuara	21	0.0461	±0.2133	0.2106	0.8
Pop 412	21	-0.1728	±0.1850	0.9105	0.4
W.C.1210	21	0.1226	±0.1962	0.8584	0.4
NEP 2	21	0.1658	±0.1865	0.8173	0.4

Table 7.3.5 Results of the correlation of percentage crude protein with seed yield in g. for the seeds on the F_1 hybrid plants of twenty one crosses of the diallel cross

n	Correlation coefficient	Standard error	't'	Probability
21	0.1276	±0.02196	0.5611	0.6

Table 7.3.6 Results of the correlation of percentage crude protein with seed yield in g. per plant for the F_2 generation of the twenty one crosses of the diallel cross

Cross No.	Cross	n	Correlation coefficient	Standard error	't'	Probability
1	Mexico 142 x Sanilac	179	-0.0992	±0.0742	1.3263	0.2
2	Mexico 142 x F.M.52	268	-0.2004	±0.0587	3.3376	0.001
3	Mexico 142 x Kabacuara	131	-0.6874	±0.0462	10.7540	0.001
4	Mexico 142 x Pop 412	139	-0.2075	±0.0814	2.4826	0.01
5	Mexico 142 x W.C.1210	129	-0.6115	±0.0553	8.7904	0.001
6	Mexico 142 x NEP2	287	0.2336	±0.0559	4.0567	0.001
7	Sanilac x F.M.52	91	-0.7653	±0.0436	11.2208	0.001
8	Sanilac x Kabacuara	113	-0.7365	±0.0430	11.5808	0.001
9	Sanilac x Pop 412	165	-0.8959	±0.0154	25.7700	0.001
10	Sanilac x W.C.1210	122	-0.8412	±0.0265	17.0522	0.001
11	Sanilac x NEP 2	159	-0.7818	±0.0309	15.7262	0.001
12	F.M.52 x Kabacuara	141	-0.1969	±0.0812	2.3680	0.02
13	F.M.52 x Pop 412	152	-0.6649	±0.0455	10.8667	0.001
14	F.M.52 x W.C.1210	152	-0.7452	±0.0361	13.6971	0.001
15	F.M.52 x NEP 2	177	-0.2818	±0.0693	3.8868	0.001
16	Kabacuara x Pop 412	112	-0.6567	±0.0542	9.0975	0.001
17	Kabacuara x W.C.1210	153	-0.0958	±0.0803	1.1831	0.2
18	Kabacuara x NEP 2	329	-0.0980	±0.0546	1.7811	0.1
19	Pop 412 x W.C.1210	204	-0.7632	±0.0293	16.7992	0.001
20	Pop 412 x NEP 2	200	-0.7179	±0.0343	14.1484	0.001
21	W.C.1210 x NEP 2	245	-0.0752	±0.0636	1.1757	0.3

Table 7.3.7 Results of the correlation of percentage crude protein with seed yield in g. per plant for the F_3 generation of six crosses

Cross No.	Cross	n	Correlation coefficient	Standard error	't'	Probability
1	Mexico 142 x Sanilac	179	-0.6429	±0.0439	11.1659	0.001
2	Mexico 142 x F.M.52	268	-0.8534	±0.0166	26.5453	0.001
3	Mexico 142 x Kabacuara	131	-0.7628	±0.0366	13.3970	0.001
4	Mexico 142 x Pop 412	139	-0.8895	±0.0177	22.7824	0.001
5	Mexico 142 x W.C.1210	129	-0.7230	±0.0421	11.7925	0.001
6	Mexico 142 x NEP 2	287	-0.6854	±0.0313	15.8897	0.001

7.4 Discussion and Conclusion

The results in Tables 7.3.1 - 7.3.7 show that yield and percentage crude protein were generally negatively correlated.

Of the varieties grown in the entire collection, and the four different variety trials, generally, high yielding varieties tended to be low in protein e.g. Kabacuara and low yielding varieties e.g. Pop 412, were high in protein. Also determinate varieties tended to have the highest protein values.

Table 7.3.3. shows that there was generally no correlation between yield and protein for the indeterminate varieties F.M.53 and Mexico 142 when grown under different levels of nitrogen. The determinate variety Sanilac and indeterminate bush variety Kabacuara, however, showed some negative correlation. This could be explained by looking at Appendix Table A5.31.1. It shows that pod numbers increased as nitrogen treatment increased for the indeterminate varieties F.M.53 and Mexico 142. However for the determinate variety Sanilac and the indeterminate bush variety Kabacuara, pod numbers increased with nitrogen, up to level 3 and then decreased. From these results it could be postulated that the determinate variety Sanilac, and the indeterminate bush variety, Kabacuara have a limited seed production or sink capacity i.e. they produce fewer flowersand set fewer pods resulting in high protein seeds. In comparison, the indeterminate varieties, F.M.53 and Mexico 142, have a larger seed production or sink capacity resulting in lower protein seeds. Thus, the sink size

appears to be established first, and this subsequently influences percent protein in the seed, based on available protein precursors in the plant.

Tables 7.3.4 and 7.3.5 show that there was no statistically significant correlation between yield and protein content in the parents and F_1 hybrid plants of the diallel cross.

Tables 7.3.6 and 7.3.7 however, show that among the F_2 and F_3 progenies, a negative correlation generally existed between yield and crude protein percentage. However, enough variation occurred to select plants that combined relatively high yields with relatively high percent crude protein. For example, some F_2 progenies of cross 6, Mexico 142 x NEP 2 look very promising. Thus, both yield and protein content might be increased simultaneously. Progress therefore seems more likely if efforts are devoted towards increased seed yield while maintaining percent crude protein near average levels rather than by selecting for high protein in seeds alone.

CHAPTER 8 RELEVANCE OF THE PRESENT STUDY TO THE IMPROVEMENT OF THE BEAN CROP

CHAPTER 8 RELEVANCE OF THE PRESENT STUDY TO THE IMPROVEMENT OF THE BEAN CROP

The improvement of the white haricot bean crop in Uganda was based upon the desirability of developing a high quality crop for export for the canning industry. A western commercial canning firm, H.J. Heinz & Co. Ltd., expressed an interest in the purchase of white haricot beans from Uganda and they specified a particular interest in higher protein varieties. The present study was therefore initiated to investigate the genetical and environmental factors affecting protein content of this type of bean. A programme of work was initiated with the objective of investigating:-

(1) the genetic variability of crude protein content in the collection of white haricot beans.

(2) the environmental effect on crude protein content

(3) the inheritance of crude protein content.

(4) the methionine content as a percentage of the crude protein content.

(5) the relationship between crude protein content and yield.

This required the use of crude protein and methionine techniques by which large numbers of plant lines could be screened quickly and efficiently. Investigations into the crude protein and methionine techniques were therefore carried out. Two techniques were considered suitable for estimating percentage crude protein content. In Uganda, the Kjeldahl - Markham technique was used for limited numbers of samples. The large collection of breeding samples were brought to Cambridge and analysed for percentage crude protein on the nitrogen auto-analyser. Both techniques were tested for the errors involved and the precision required.

With regard to the methionine techniques, two methods were investigated, those of Bolinder (1968) and Kelly et al (1970). The aim was to find which technique was most suitable for screening the large numbers of samples from the breeding programme. Difficulties arose with the Bolinder method because some of the amino acids used in the media were not of sufficient purity. The Kelly method was therefore chosen since it gave reasonable consistency and sufficient quantitative accuracy.

These protein and methionine techniques were then used to assess the genetic variation in the protein and methionine content of the collection of white haricot beans. The percentage crude protein content of the different varieties of the entire collection varied between 20.2% and 29.7%. The methionine content varied between 0.61 and 1.97 mg. available methionine/g. bean. Methionine expressed as a percentage of the protein content indicated that in general, high protein varieties had high methionine content and low protein varieties had a low methionine content.

From this collection of white haricot beans, twenty five varieties were selected on the basis of their having some or all of the desirable characters for canning. They were then assessed in Variety Trials in four different seasons over two years in order to estimate the environmental effect on percentage crude protein and methionine content. The percentage crude protein results over these four different seasons showed that there was a considerable variety x season interaction, indicating that the environment had a considerable effect on protein content. The methionine content of each variety however remained the same over four different seasons indicating no environmental effect on methionine content.

Because of this environmental effect on protein content it was decided to consider the effect of different levels of fertiliser treatment on the protein and methionine content. Four varieties were assessed under five different levels of fertiliser. The results indicated a significant variety x fertiliser treatment interaction for percentage crude protein content but not for methionine content. With the indeterminate vine varieties, the effect of fertiliser on the protein content was not as great as the effect with the more determinate varieties. This could be explained by the fact that with the indeterminate varieties, the increases in nitrogen are used for vegetative growth and branching i.e. increases in fertiliser caused a greater degree of indeterminancy, and to some extent an increase in yield. With the determinate variety there is vegetative growth to a lesser degree as the nitrogen is increased, so that the nitrogen is channelled into the seeds in the form of protein.

This preliminary study of the effect of environment on protein content indicated that for the protein inheritance study, it was important to grow all generations in one season and under as near to possible uniform environmental conditions.

A quantitative genetical approach to the study of protein inheritance was adopted on the assumption that protein content was under genetical control and was not inherited in a simple Mendelian fashion. An analysis of the diallel set of crosses between homozygous lines, their F_1 recombinants, and respective F_2 progeny following selfing was adopted for this study. The adequacy of the diallel model chosen was first tested and proved suitable. Analyses of variance were then carried out to test the significance of some of the genetical components of variation and

also the validity of some of the subsidiary assumptions underlying the simple model.

The protein results showed that:-

(1) the protein content of the Fo hybrid single seed was close to the protein content of the maternal parent indicating a strong maternal effect in the Fo seed.

(2) the protein content of seeds borne on the F_1 hybrid plant was close to the protein content of the low protein parent.

(3) the protein content results of seeds borne on the F_2 generation plants were normally distributed for each cross except crosses 6 and 7 where the distribution were skewed.

(4) similarly, the protein content of the plants of the F_3 generation crosses were normally distributed with the mean towards the low protein parent.

These results indicate that low protein content is dominant. Similarly the Wr/Vr graph of the diallel analyses for the F_1 hybrid plant shows that the low protein varieties have the dominant genes. The distinction is not so clear for the Wr/Vr graph of the diallel analysis of the F_2 generation plants. However it could be deduced that protein content is controlled by a series of genes with minor effects which are additive and which show a dominance relationship for low protein in some of the loci.

A further analysis of the protein results of the diallel cross generations shows that narrow sense heritability is low. Since heritability in the narrow sense expresses the fraction of the range of phenotypic values of the parents that one expects to recover in the offspring, then for protein in beans, it can be

postulated that one would expect a gain of about 5-30 per cent depending on the selection pressure.

However, protein content alone cannot just be considered. It is important to consider other characters - particularly yield and its relationship with protein content. The results of the protein/yield relationship indicated a dependance on the growth habit of the variety. The results indicate that the determinate varieties have a limited seed production or sink capacity i.e. they produce fewer flowers and set fewer pods resulting in high protein seeds. In comparison, the indeterminate vine varieties have a longer seed production or sink capacity resulting in lower protein seeds. Thus, the sink size appears to be established first, and this subsequently influences percent protein in the seed, based on available protein precursors in the plant. The results indicate that generally a negative correlation exists between yield and percentage crude protein content.

However, enough variation does occur to select plants that combine relatively high yields with above average percent crude protein content. Thus both yield and protein content might be increased simultaneously. Progress therefore seems more likely if efforts are devoted towards increased seed yield while maintaining percent crude protein near or above average levels rather than by selecting for high protein in seeds alone.

The results and guidelines from this study have been applied to a particular cross from the diallel. The F_3 generation of cross 6, Mexico 142 x NEP 2 was very promising. High yielding plants with above average protein content were selected and are now in the F_5 stage of development. It is hoped that these selections will be of direct use for developing a suitable canning crop for export from Uganda, or for use in a future breeding programme.

BIBLIOGRAPHY

Allard, R.W. (1956) Estimation of prepotency from lima bean diallel cross data. Agron. J. Vol. 48 : 537-543.

Anderson, A.L., Down, E.E., Whitford, G. (1960) The Sanilac Pea Bean - its history, development and characteristics. The Quarterly Bulletin, Vol. 43 No. 22 : 214-236.

Ashworth, U.S., Seals, R., Erb, R.E. (1960(a)) An improved procedure for the determination of milk proteins by dye-binding. J. Dairy Sci. Vol. 43 : 614-623.

Ashworth, U.S., Chaudry, M.A. (1960(b)) Dye-binding capacity of milk proteins for amido black 10B and Orange G. J. Dairy Sci. Vol. 45 : 952-957.

Bender, A.E., Doell, B.H. (1956) Biological evaluation of proteins : a new aspect. J. Nutr. Vol. 11 : 140-157.

Bolinder, A.E. (1968) Plate assay methods for amino acids (cyclostyled).

Boulter, D. (1972) Private communication.

Boyde, D. (1971) Private communication.

Boyne, A.W., Price, S.A., Rosen, G.D., Stott, J.A. (1967) Protein quality of feeding stuffs. 4. Progress report on collaborative studies on the microbiological assay of available amino acids. Brit. J. Nutr. Vol. 21 : 181-206.

Bressani, R., Elais, L.G., Valiente, A.T. (1963) Effect of cooking and of amino acid supplementation on the nutritive value of black beans (Phaseolus vulgaris) Brit. J. Nutr. Vol. 17 : 69-78.

Bressani, R., Flores, M., Elias, L.G. (1973) Acceptability and value of food legumes in the human diet. Report by Centro Internacional de Agricultura Tropical (C.I.A.T.)

Burton, G.W. (1951) Quantitative inheritance in pearl millet (Pennisetum glaucum). Agron. J. Vol. 43 : 409-417.

Cochran, W.G., Cox, G.M. (1957) Experimental Designs. Wiley International Edition.

Darbre, A., Islam, A. (1968) Gas Liquid Chromatography of Trifluoro-acetylated Amino Acid Methyl Esters. Biochem. J. Vol. 106 : 923-925.

Darlington, D.C., Ammal, E.K. (1945) Chromosome Atlas. George Allen and Unwin Ltd.

Davis, B.J. (1964) Disc Electrophoresis II : Method and application to the human serum proteins. Ann. N.Y. Acad. Sci. Vol. 121 : 404-427.

Down, E.E., Anderson, A.L. (1956) Agronomic use of an X-ray induced mutantand. Science. Vol. 124 : 223-224.

Ford, J.E. (1960) A microbiological method for assessing the nutritional value of proteins. Brit. J. Nutr. Vol. 14 : 485-497.

Ford, J.E. (1962) A microbiological method for assessing the nutritional value of proteins. 2. The measurement of 'available' methionine, leucine, isoleucine, arginine histidine, tryptophan, and valine. Brit. J. Nutr. Vol. 16 : 409.

Frankel-Contrat, H., Cooper, M. (1944) The use of dyes for the determination of acid and basic groups in proteins. J. Biol. Chem. Vol. 154 : 239-246.

Griffing, B. (1956) The concept of general and specific combining abilities in relation to diallel crossing systems. Australian J. Biol. Sci. Vol. 9 : 462-493.

Hamblin, J., Woolfe, J. (1971) Private communication.

Hayman, B.I. (1954 (a)) The analysis of variance of diallel tables. Biometrics. Vol. 10 : 235-244.

Hayman, B.I. (1954(b)) The theory and analysis of diallel crosses. Genetics. Vol. 39 : 789-809.

Hayman, B.I. (1960) The separation of epistatic from additive and dominance variation in generation means. : Genetica. Vol. 31 : 133-146.

Hayman, B.I. (1963) Notes on diallel cross theory: 571-578 In statistical genetics and plant breeding (ed. by W.D. Hanson and H.F. Robinson). National Academy of Sciences, National Research Council, Washington, D.C.

Henderson, C.R. (1952) Specific and general combining ability. In Heterosis (ed. by J.W. Gerves) : 352-370. Iowa State College Press, Ames, Iowa.

Horn, M.J., Blum, A.E., Womark, M., Gersdorff, C.E.F. (1952) Nutritional evaluation of food proteins by measuring availability of amino acids to micro-organisms. J. Nutr. Vol. 48 : 231-241.

Horn, M.J., Blum, A.E., Womark, M. (1954) Availability of amino acids to micro-organisms. II. A rapid microbial method of determining protein value. J. Nutr. Vol. 52 : 375-381.

Inglis, L., Edman, C. (1970) Gas chromatography procedures for the analysis of proteins. Anal. Biochem. Vol. 37 : 73.

Jinks, J.L. (1954) The analysis of continuous variation in a diallel cross of Nicotiana rustica varieties. Genetics. Vol. 39 : 767-788.

Jinks, J.L. (1956) The F_2 and backcross generations form a set of diallel crosses. Heredity. Vol. 10 : 1-30.

Jinks, J.L., Hayman, B.I. (1953) The analysis of diallel crosses. Maize Genetics Coop, News Letter. Vol. 27 : 48-54.

Jinks, J.L. Mather, K. (1955) Stability in development of heterozygotes and homozygotes. Pro. Roy. Soc. B. Vol. 143 : 561-578.

Jones, R.M. (1965) Analysis of variance of the half diallel table. Heredity. Vol. 20 : 117-121.

Juo, P., Strotzky G., (1970) Proteins of seeds during germination. Canadian J. of Bot. Vol. 48 : 1347-1350.

Kelly, J.F., Firman, A., Adams, H.L. (1970) Microbiological methods for the estimation of methionine content of beans. Presented at the 10th Dry Bean Res. Conf. Davis,California.

Kelly, J.F. (1971) Genetic variation in the methionine levels of mature seeds of common beans (Phaseolus vulgaris L.) J. of the Amer. Soc. for Hort. Sci. Vol. 96, No. 5 : 561-563.

Khai, F.S., Pleshkov, B.P. (1964) Changes in the composition of proteins of bean seeds as a function of the conditions of nutrition. Translated from Doklady Akademi Nank USSR. Vol. 162 No. 1 : 215-218.

Kidder, G.W., Dewey, V.C. (1957) Biochemistry and Physiology of Protozoa. (ed. by A. Lwoff). Vol. 1 : 323, Academci Press, New York.

Knight, R.L. (1948) Dictionary of Genetics. Waltham Mass : Chronica Botanica Co.

Kodi^{cek} E., Mistry, S.P. (1957) Biological activity for the rat of strepogenin like peptides in partially hydrolysed protein. Proc. Nutr. Soc. Vol. 16 : 13-14.

Kuenzler, A. (1957) A report submitted to the Tanganyikan Farmers' Association on the prospects for a canning bean industry (typed).

Leakey, C.L.A. (1969) Private communication.

Leakey, C.L.A. (1970(a)) The selection and improvement of white haricot beans for canning. In Crop Improvement in East Africa (ed. by C.L.A. Leakey). Tech. Comm. 19 : 116-119 Commonwealth Bureau of Plant Breeding and Genetics.

Leakey, C.L.A. (1970(b)) Draft project proposal for the development of white haricot beans as an export crop from Uganda (cyclostyled).

Leakey, C.L.A. (1971) Private communication.

Leakey, C.L.A. Stabursvik, A. (1970) Scope for breeding for improved protein content and quality in dry seed beans in Uganda. Ann. Rep. of the Bean Coop. Vol. 13 : 61-62.

Leakey, C.L.A., Stabursvik, A. (1971) Private communication.

Leffel, R.C., Hanson, W.D. (1961) Early generation testing of diallel crosses of soybeans. Crop Sci. Vol. 1 : 169-174.

Lele ji, O.I., Dickson, M.H., Crowder, L.V., Bourke, J.B. (1972) Inheritance of crude protein percentage and its correlation with seed yield in beans, Phaseolus vulgaris L. Crop Sci. Vol. 12 : 168-171.

Lindegren, D. (1971) Physical and nondestructive screening techniques (cyclostyled).

Lowry, O.H., Rosebrough, Farr, A.L., Randell, R.J. (1951) Protein measurement with Folin phenol reagent. J. Biol. Chem. Vol. 193 : 265-275.

Luse, R.A. (1970(a)) Mass screening for specific amino acids by nuclear chemical, biological, and enzymatic techniques. Reprint from "Improving Plant Protein by Nuclear Techniques" I.A.E.A.

Luse, R.A. (1970(b)) Background of the F.A.O./I.A.E.A. coordinated programme on the application of nuclear techniques to the improvement of plant protein. Reprint from Sabrao Newsletter 2 (1) : 71-72.

Macartney, J.C. (1960) The history of the Canning Bean Industry in Tanganyika (typed).

Macartney, J.C. (1966) Tanzania Dept. Agric. Northern Region Res. Cent., Res. Note, No. 3 (cyclostyled).

Mahmud, I., Kramer, H.H. (1951) Segregation for yield, height, maturity following a soybean cross. Agron. J. 43 : 605-609.

Mather, K. (1949) Biometrical Genetics. London Mathuen & Co.

Meiners, J.P., Litzenberger, S.C. (1970) Current status of Breeding Food Legumes for Nutritional Improvement (typed).

Moh, C.C. (1969) Seed coat colour changes induced by ethyl methane sulphonate in the common bean (Phaseolus vulgaris L.) Mutation Res. Vol. 7 : 469-471 (Fig. 1 right hand side of picture is infact NEP 2).

Moh, C.C. (1971) Mutation breeding in seed coat colours of beans (Phaseolus vulgaris L.) Euphytica Vol. 20 : 119-125.

Moore, S., Stein, W.H. (1951) Chromatography of amino acids on sulphonated polystyrene resins. J. Biol. Chem. Vol. 192 : 663-681.

Mossberg, R. (1971) Evaluation of protein quality and quantity by dye-binding capacity : a tool in plant breeding. New approaches to breeding for improved plant protein : 151-160 International Atomic Energy Agency, Vienna.

Official methods of Analysis of the Association of Official Agricultural Chemists. 1933-1955.

Outen, G.E., Tilley, J.M.A., Wilson, R.F. (1966) Estimation of protein in dried herbages using the dyestuff Orange G. J. Sci. Fd. Agric. Vol. 17 : 285-286.

Pilcher, H.L., Williams, H.H. (1954) Microbiological evaluation of protein quality. II. Studies of the responses of Tetrahymena pyriformis W to intact proteins J. Nutr. Vol. 53 : 589-599.

Pomeranz Y., (1965) Evaluation of factors affecting the determination of nitrogen in soya products by the biuret and orange G dye binding method. J. Fd. Sci. Vol. 30 : 307-311.

Reisfeld, R.A., Lewis V.J., Williams, D.E., (1962) Disc electrophoresis of basic proteins and peptides on polyacrylamide gel. Nature Vol. 195 : 281-283.

*Rockland, L.B., Dunn, M.S., (1949) Food Technol. Vol. 3 : 289.

Rosen, G.D., Fernell, W.R. (1954) Microbiological Assay of Protein Quality. 2. Comparison of nutritive values of Proteins. Proc. Nutr. Soc. Vol. 13 : 19-20.

Rosen, G.D., Fernell, W.R. (1956) Microbiological evaluation of protein quality with Tetrahymena pyriformis W. 2. Relative nutritive values of proteins in food stuffs. Brit. J. Nutr. Vol. 10 : 156-169.

Rutger, J.N. (1968) Variation in protein content and its relation to others characters in beans (Phaseolus vulgaris L.). Agron. Abstr.: 20

Rutger J.N. (1971) Variation in protein content and its relation to other characters in beans, Phaseolus vulgaris L. Rept. Dry Bean Res. Conf. Davis, Calif. Vol. 10 : 59-60.

Silbernagel, M.J. (1968) Bean protein studies. Bean Impr. Coop. Vol. 11 : 45.

Silbernagel, M.J. (1969) Bean protein improvement. Bean Impr. Coop. Vol. 12 : 33.

Silbernagel, M.J. (1971) Bean protein improvement work by U.S.D.A. Bean and Pea investigations. Talk presented to 10th Annual Dry Bean Res. Conf. Davis, Calif.

Spackman, D.H., Stein, W.N., Moore, S. (1958) Automatic Recording Apparatus for use in the chromatography of amino acids. Anal. Chem. Vol. 30 : 1190-1205.

Stokes, J.L., Gunnes, M., Dwyer, I.M., Caswell, M.C. (1945) Microbiological Methods for the determination of amino acids. II. A uniform assayfor the ten essential amino acids. J. Biol. Chem. Vol. 160 : 35-49.

Torton, J., Whitaker, J.R. (1964) Evaluation of the biuret and dye-binding methods for the protein determination in meats. J. Fd. Sci. Vol. 29 : 168-174.

Udy, D.C. (1956) Estimation of protein in wheat and flour by ion-binding. Cereal Chem. Vol. 33 : 190-197.

*Ussary and Gehrke (1969) Proc. Technicon International Congress in Adv. Automated Anal. Vol. 10 : 89-94.

Warner, J.N. (1952) A method of estimating heritability. Agron. J. Vol. 44 : 427-430.

Wearden, S. (1964) Alternative analyses of the diallel cross. Heredity. Vol. 19 : 669-680.

Whitehouse, R.N.H., Thompson, J.B., Ribeiro, M.A.M. (1958). The use of a diallel cross analysis in yield prediction in wheat. Euphytica. Vol. 7 : 147-169.

Wood, D.R., Waide, J.J., Cole, C.V., Ross, C. (1969) Bean seed protein studies. Bean Impr. Coop. Vol. 12 : 39.

Yates, F. (1947) The analysis of dates from all possible reciprocal crosses between a set of parental lines. Heredity. Vol. 1 : 287-301.

Yohe, J.M., Swindell, R.E., Watt, E.E., Bashandi, M.M.H., Sechler, D.T., Poetrlema, J.M. (1972) Evaluation of mungbean (Phaseolus aurens) Strains at Colombia Missouri. Dept. of Agronomy, Miscellaneous Publication.

*indicates not read in the original

PLATES

PLATE 1 Variety, Mexico 142

PLATE 2 Variety, Sanilac

PLATE 2

PLATE 3 Variety, F.M.52

PLATE 4 Variety, Kabacuara

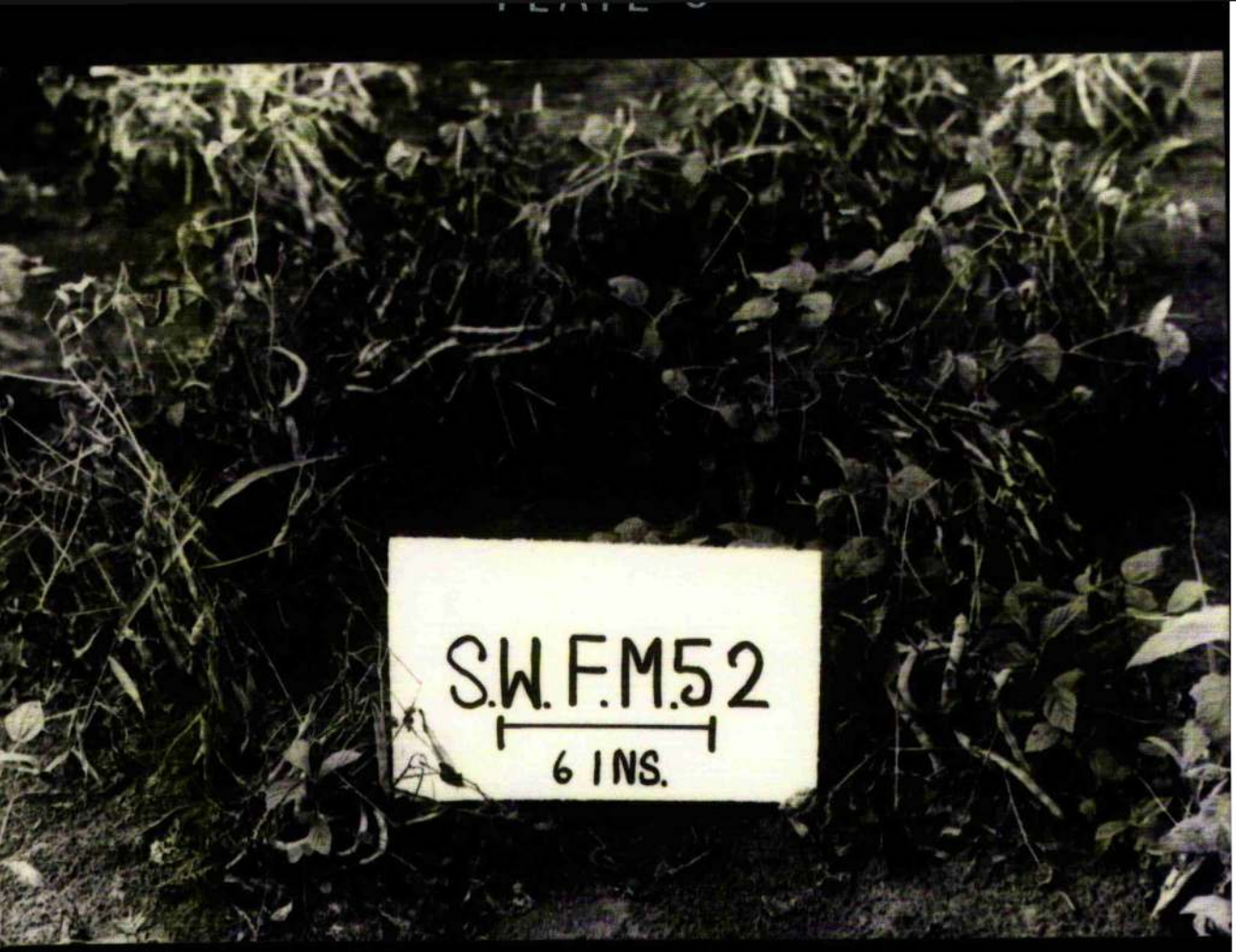


PLATE 4

PLATE 5 Variety, Pop 412

PLATE 6 Variety, W.C. 1210

PLATE 6

PLATE 7 Variety, NEP 2


NEP. 2
6 INS.

APPENDICES

List of Tables in the Appendix

For each table, the first digit indicates the chapter number, the second digit the section in the chapter, the third digit the table number.

List of Figures in the Appendix

For each figure, the first digit indicates the chapter number, the second digit the section in the chapter, the third digit, the figure number.

APPENDIX TO SECTION 3.2 of CHAPTER 3

Appendix Table A3.21.1 Percentage crude protein values and coefficient of variation values (C.V.) for testing the Analytical Technique, Experiment 1

Variety : Verdon

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
27.3000		28.7875		27.3875	
27.2125	0.58	28.8750	0.25	27.4750	0.26
27.4750		28.8750		27.4750	
27.5625		28.9625		27.5625	

Variety : NEP 2

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
28.8750		27.2125		27.3000	
28.7875	0.15	27.3875	0.04	27.6500	1.08
28.8750		27.3875		28.0000	
28.8750		27.4750		27.8250	

Variety : Mexico 184

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
26.8625		29.4875		26.8625	
26.6875	0.31	30.2750	1.84	26.6875	0.31
26.6875		30.7125		26.6875	
26.7750		30.6250		26.7750	

Appendix Table A3.21.2 Percentage crude protein values and coefficient of variation values (C.V.) for testing the Grinding Technique, Experiment 2

Variety : Verdon

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
25.2875		26.8625		26.1625	
25.7000		27.0375		26.3375	
25.2875	0.24	26.9500	0.42	26.4250	0.41
25.3750		27.1250		26.3375	
25.2875		-		26.4250	

Variety : NEP 2

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
29.2250		27.2125		23.5375	
29.3125		27.3000		23.4500	
29.4000	0.25	27.0375	0.37	22.8375	1.27
29.3125		27.1250		23.4500	
29.2250		27.2125		23.1000	

Variety : Mexico 184

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
28.7875		23.8000		25.2000	
28.8750		23.5375		25.2875	
28.9625	0.25	23.5375	0.48	25.2875	0.39
28.8750		23.6256		25.3750	
28.9625		23.7125		25.4625	

Appendix Table A3.21.3 Percentage crude protein values and coefficient of variation values (C.V.) for testing the Pod Sampling Technique, Experiment 3

Variety : Verdon

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
(3)29.5313		(3)28.0438		(3)22.6625	
(2)26.8625	5.00	(2)30.1438	5.10	(2)24.5438	15.44
(1)27.4758		(1) -		(1)30.3188	

Variety : NEP 2

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
(3)30.0563		(3)25.9000		(3)24.1063	
(2)27.1688	5.22	(2)27.2125	6.18	(2)27.8688	9.44
(1)29.3563		(1)29.2688		(1)28.9625	

Variety : Mexico 184

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
(3)24.1063		(3)25.1563		(3)28.6563	
(2)24.8063	2.48	(2)28.6125	9.77	(2)31.8063	6.82
(1)25.3313		(1)30.5813		(1)32.6813	

Key

(1) = early formed seeds

(2) = intermediate formed seeds

(3) = late formed seeds

Appendix Table A3.21.4 Percentage crude protein values and coefficient of variation values (C.V.) for testing the Single Plant Sampling Technique, Experiment 4(a)

Variety : Verdon

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
26.3200		27.2125		27.3438	
26.1275	0.42	27.2125	0.41	27.3875	1.16
26.3200		27.4050		26.8188	

Variety : NEP 2

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
28.7350		27.6500		27.8250	
28.8750	0.25	27.3875	1.56	27.8250	0.73
28.7875		26.8188		27.4750	

Variety : Mexico 184

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
25.4625		26.3025		28.9625	
25.2875	0.71	26.8188	1.02	28.8750	0.55
25.1010		26.4250		28.6563	

Appendix Table A3.21.5 Percentage crude protein values and coefficient of variation values (C.V.) for testing the Single Plant Sampling Technique, Experiment 4(b)

Variety : Verdon

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
25.9700		27.4050		26.1275	
24.7450	2.77	27.2125	3.86	25.1010	4.37
25.9700		25.5325		27.3875	

Variety : NEP 2

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
26.3025		25.9175		28.4375	
28.7350	4.70	25.3225	1.16	23.2925	9.93
26.8100		25.6375		25.9700	

Variety Mexico 184

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
28.1050		26.4250		28.4725	
25.2000	7.12	26.1625	0.50	27.6500	5.83
28.9100		26.3200		25.4100	

Appendix Table A3.21.6 Percentage crude protein values and coefficient of variation values (C.V.) testing the Single Plant Sampling Technique, Experiment 4(c)

Variety : Verdon

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
26.8100		27.2125		27.6500	
25.4625	2.58	26.3200	1.67	27.4050	0.80
26.1275		26.8188		27.2125	

Variety : NEP 2

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
28.7350		27.3000		28.9100	
27.4750	2.71	27.5625	3.02	27.4750	3.86
27.3875		28.8750		26.8188	

Variety : Mexico 184

Rep 1		Rep 2		Rep 3	
% crude protein	C.V.	% crude protein	C.V.	% crude protein	C.V.
26.4250		27.4050		26.3025	
24.5438	3.72	26.6875	3.81	26.8188	4.17
25.3225		25.4100		28.4725	

Appendix Table A3.21.7 Percentage crude protein values and coefficient of variation values (C.V.) for testing the Bulk Sampling Technique, Experiment 5

Variety : Verdon

Replicate	% crude protein	C.V.
1	26.8188	
2	28.3938	2.91
3	27.3438	

Variety : NEP 2

Replicate	% crude protein	C.V.
1	28.7438	
2	26.3375	4.39
3	27.8250	

Variety : Mexico 184

Replicate	% crude protein	C.V.
1	27.7813	
2	27.2125	2.09
3	26.6438	

APPENDIX TO SECTION 3.22 of CHAPTER 3

Appendix Table A3.22.1 Experiment (i) Twenty different varieties put through the analyser six times

Variety No.	Day 1			Day 2			S.D.	Mean S.D.	% coefficient of variation
	1	2	3	1	2	3			
1	30.8	30.9	31.0	30.3	30.1	30.5	0.33		
2	27.5	27.4	27.5	26.9	26.3	27.2	0.43		
3	25.6	25.5	25.5	25.0	24.6	25.2	0.35		
4	26.1	26.1	26.1	25.5	24.9	25.8	0.44		
5	24.0	23.7	23.8	23.3	22.6	23.6	0.45		
6	23.4	23.3	23.4	22.7	22.1	23.1	0.47		
7	28.4	28.6	28.8	28.1	27.2	28.4	0.45		
8	33.2	33.2	33.1	32.6	31.8	33.2	0.49		
9	28.5	28.5	28.6	28.0	27.4	28.5	0.43		
10	26.3	26.5	26.6	25.8	25.6	26.2	0.36	0.36	1.3
11	23.0	23.0	23.0	22.6	22.2	22.9	0.30		
12	22.5	22.5	22.5	22.2	21.7	22.4	0.26		
13	26.6	26.5	26.6	26.3	25.7	26.9	0.37		
14	24.8	24.8	24.8	24.4	24.0	24.7	0.30		
15	27.3	27.5	27.6	27.1	27.0	27.6	0.25		
16	30.2	30.3	30.4	29.8	29.6	30.3	0.35		
17	28.7	29.0	29.0	28.5	28.5	29.0	0.23		
18	28.6	28.8	28.7	28.1	28.1	28.7	0.29		
19	28.6	28.7	28.9	28.3	28.4	28.7	0.20		
20	26.9	27.0	27.1	26.4	26.2	27.6	0.25		

Appendix Table A3.22.2 Experiment (ii) Repeated digestions of one grinding of ten beans of the variety Provider (V1630)

% crude protein	Mean	Standard Deviation	Coefficient of variation %
27.2			
27.5			
27.5			
27.9	27.5	0.49	1.78
28.5			
27.3			
26.9			

APPENDIX TO SECTION 3.3 of CHAPTER 3

Bolinder's Method

In this method, total methionine is measured and the sample has first to be hydrolysed before assay. Hydrolysis is with 3-N HCl in sealed evacuated glass ampoules at 110°C for 8-24 hours. A 50 mg. sample with 20 ml. of 3N HCl is used. The ampoules are then removed from the oven and allowed to cool to room temperature for direct neutralisation. The sample is then ready for assay. The assay procedure was as follows.

1) Maintenance of the Test Organism

The organism used, Pediococcus cerevisiae A.T.C.C. No. 8081 was maintained on a stab culture on Bacto Micro Assay Culture (see Appendix Table A3.3.1).

2) Preparation of the inocula media

The inocula media was used when cultivating the test organism for preparation of the plate inoculum. The composition of the inoculum medium is given in Appendix Table A3.3.2, and consists of a complete amino acid medium, agar being excluded. The inoculum medium was prepared by placing 5ml. portions together with a glass bead, in centrifuge tubes and sterilising for 10 minutes at 120°C. If the cotton plugged tubes were covered with aluminium foil, the inoculum medium could be stored for over six months when kept at +4°C. Only a minor portion of the tubes, ready for immediate use, were kept at room temperature.

3) Preparation of suspension media

The suspension media was used for washing and diluting the inocula to the desired turbidity for use as the plate inocula. The suspension media consists of the appropriate single strength assay medium (the complete medium excluding

the amino acid being assayed) together with Tween 80, but excluding the agar. The suspension media were not stored ready for use but were prepared from the double strength assay media, each time a plate was made. Suitable volumes of the suspension media were sterilised simultaneously with the agar plate media.

4) Preparation of the Plate

The plate used consisted of a chromium plated brass frame lying between two sheets of ordinary window glass. The dimension of the plate used was 20 x 40 cm. For sterilisation, the plate was wrapped in paper and kept overnight in an oven at 100°C.

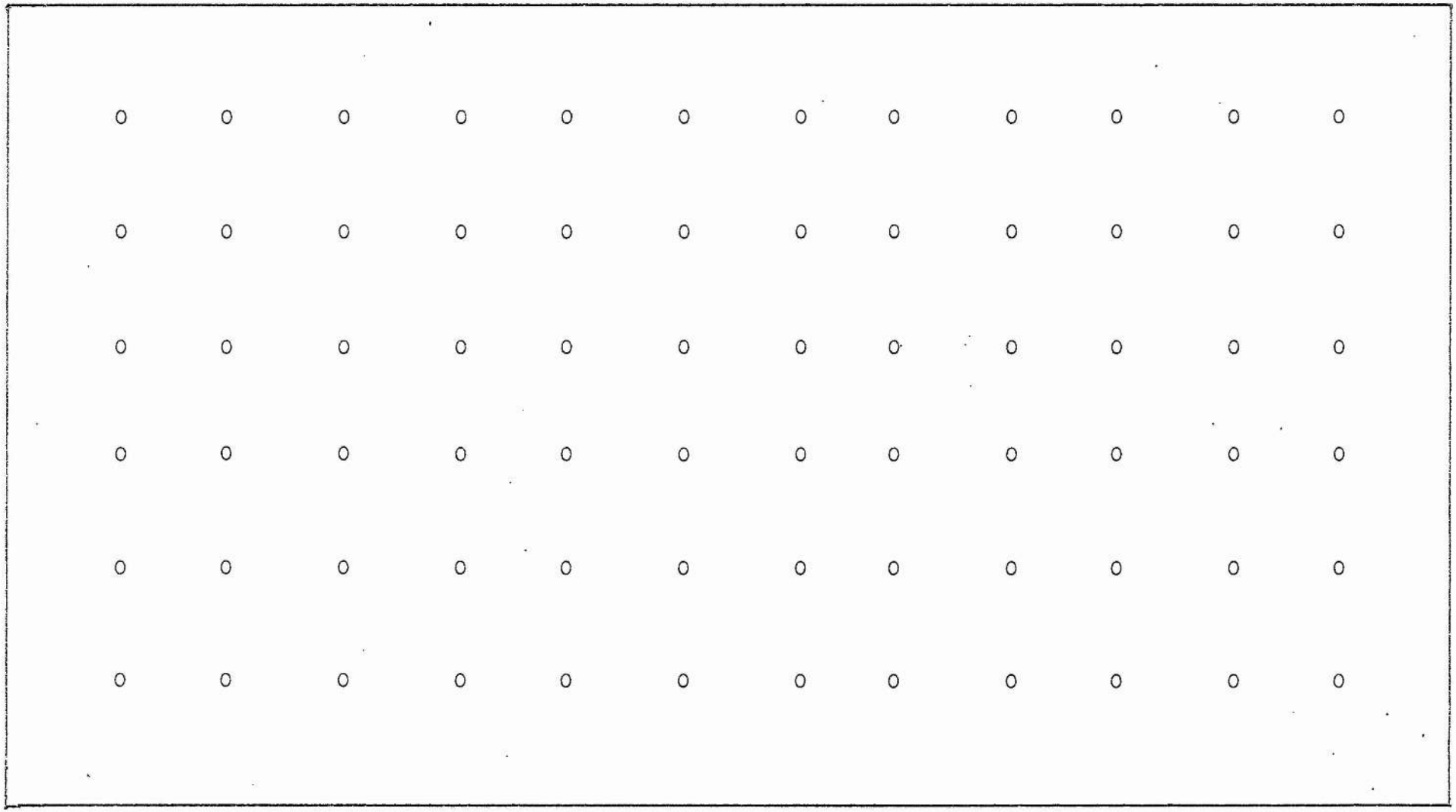
A cup scheme was drawn on cardboard and placed under the plate for guidance when punching out the cups and also for rapid pipetting of solutions into the cups. A diagram of the cup scheme used is shown in Appendix Fig. A3.3.1. The plate was kept on a carefully levelled surface.

The plate inoculum (containing an optically standardised suspension of the test organisms) was then mixed with the assay medium containing agar (see Appendix Table A3.3.2 for the contents of the media). The medium was then poured evenly onto the plate, flamed to get rid of air bubbles, and allowed to cool for half an hour. Six millimetre holes were then punched in the agar with the specially designed agar hole punching apparatus. The standard and test solutions (for details see Appendix Tables A3.3.3) were then dropped into cups in 30µl. aliquots using a syringe. The plate was then covered with a sheet of glass, left at room temperature for ten minutes, then incubated overnight at 37°C.

The bacterial growth zones could then be measured directly using calipers with needle attachments or the plate could be photographed.

The zone diameters could then be read off the standard graph to obtain the methionine concentrations (The results were expressed as µg. methionine per ml. of solution.

APPENDIX FIG. 3.3.1 A diagram of the cup scheme used (72 holes)



APPENDIX TABLE A3.3.1 Composition of the Bacto Micro Assay Culture Agar (Difco)

Component	Amount in g.
Bacto Yeast Extract	20
Proteose Peptone No. 3 (Difco)	5
Bacto Dextrose	10
Monopotassium phosphate	2
Sorbitan mono oleate complex (Tween 80)	0.1
Bacto Agar (Difco)	10

Make up to 100ml. with distilled water

APPENDIX TABLE A3.3.2 Composition of the complete amino acid medium used as the Plate assay medium (a), suspension medium (a) and inoculum medium

Component	Amount
Glucose	20g.
Sodium acetate, $3.H_2O$	3g.
Trisodium citrate, $2.H_2O$	20g.
Dipotassium phosphate	3g.
Sodium chloride	1g.
Ammonium sulphate	3g.
DL - Alanine	300mg.
L - Arginine	500mg.
L - Asparagine	200mg.
DL - Aspartic acid	200mg.
L - Cystine - hydrochloride	200mg.
L - Cystine	200mg.
L - Glutamic acid	500mg.
Glycine	300mg.
DL - Histidine hydrochloride	200mg.
DL - Iso leucine	300mg.
DL - Leucine	300mg.
L - Lysine hydrochloride	200mg.
DL - Methionine	200mg.
DL - Phenylalanine	200mg.
L - Proline	100mg.
DL - Serine	200mg.
DL - Threonine	200mg.
DL - Tryptophan	200mg.

L - Tyrosine	100mg.
DL - Valine	300mg.
Adenine sulphate	10mg.
Guanine hydrochloride	10mg.
Uracil	10mg.
Xanthine	10mg.
Thiamine hydrochloride	1mg.
Riboflavin	1mg.
Niacin	1mg.
Calcium pantothenate	1mg.
Pyroxidine hydrochloride	1mg.
Pyridoxal hydrochloride	1mg.
p-Amino benzoic acid	500µg.
Biotin	10µg.
Folic acid	10µg.
Folinic acid (leucovorin)	10µg.
Calcium chloride, $2.H_2O$	100mg.
Magnesium sulphate, $7.H_2O$	500mg.
Manganese sulphate, $4.H_2O$	150mg.
Ferrous sulphate, $7.H_2O$	50mg.
Tween 80[b]	200mg.
Bacto Agar[c]	20mg.

The amounts have reference to 1000ml.

For the assay of methionine, all amino acids except methionine were added.

(a) The pH of the medium was adjusted to 6.8.

(b) Polyoxyethylene sorbitan monooleate - added just before sterilisation.

(c) Only used in the plate assay medium - added just before sterilisation.

APPENDIX TABLE A3.3.3 Preparation of the L-methionine standard solutions

A strong stock solution containing 1000µg of L-methionine/ml. was prepared by weighing 100.0 ± 0.1 mg. of L-methionine and dissolving it in about 70ml. of sterile distilled water. The pH was adjusted to 6.8 ± 0.1 with NaOH and the volume made up to 100ml. with sterile distilled water in a volumetric flask. The following dilutions were then made with sterile distilled water : 1, 3, 10, 30 and 100µg/ml. and stored at -20°C in a number of small flasks.

Results

Most unsatisfactory results were obtained. The plate assay procedure was repeated many times with varying experimental conditions to determine which stage of the technique was at fault. A typical result is shown in Appendix Fig. A3.3.2. It can be seen that the bacterial growth developed round the cup holes but spread irregularly round the holes. This type of growth was impossible to measure and would have given very unreliable results. Different stages in the experimental procedure were therefore repeated and tested. The different experimental conditions which were thought may have given variable and obscure results are listed below:-

(1) The pH of the medium, sample solutions and standard solutions.

(2) The mixing of the medium with the inocula before pouring the plate.

(3) The temperature of the solutions dropped into the cups.

(4) The incubation of the plate

Each of these conditions is considered in turn.

APPENDIX FIG. 3.3.2 A typical result found using the standard methionine solutions

(1) The pH of the medium, sample solutions and the standard solutions.

On testing the pH of the standard and test solutions, it was found to vary between 6.4 and 7.3. The pH of the medium remained at 6.8. The pH of the standard and test solutions were therefore corrected to 6.8 before application to the plate. However, no real difference was shown in the growth responses.

(2) The mixing of the inocula with the media before pouring the plate.

An even spread of inocula in the media was essential before pouring the plate. This had to be done carefully in order to avoid air bubbles and rapid cooling of the agar media before pouring. Again, no real difference was observed in the growth response after ensuring even mixing of the inocula of the media.

(3) The temperature of the solutions dropped in the cups.

All sample and standard solutions were kept at the same temperature before applying them to the plate. This was because the rate of diffusion of the growth substance and growth rate of the test organism are temperature dependant. However, there was no improvement in the growth rate of the organism.

(4) The volume of solutions dropped into the cups.

The amount of solution applied in a cup had to be adjusted to match the cup diameter, or more precisely, the cup volume.

The thickness of the agar layer was approximately 2mm. and the diameter of the cups 6mm. Different aliquots of 10µl, 20µl, 30µl, 40µl, 50µl were tried.

30µl was found to be the most suitable volume to use. It occupied approximately 50% of the volume of the cup and gave a better growth response. 10µl gave no growth because the solution dried up in the incubator; 50µl was too much - in some cases it overflowed from the cup causing growth responses of neighbouring cups to overlap.

(5) The incubation of the plate.

Only a bacteriological incubator was available for incubation. This was not very satisfactory because the temperature in the incubator varied so that the rate of temperature increase over the whole plate was not constant. An incubation room with circulation of air was really desirable. However, to reduce the effect of uneven increase of temperature over the plate, the plate was pre-incubated for one or two hours at 37°C (the cups had already been cut out). The warm plate was then taken out immediately before applying the solutions, and the solutions were applied as quickly as possible (10-15 minutes) and then the plate was immediately returned to the incubator.

Another effect of this preincubation of the plate was that the test organisms started forming growth zones immediately after the solutions had been distributed into the cups. The rate of diffusion increased. It was hoped that these factors would help to improve the appearance of the growth zones by giving a denser growth and sharp edges.

There was certainly a denser growth but the boundaries were very irregular.

Discussion and conclusion

Some blanks were found in the assays, some growth zones with fuzzy edges and in some cases background growth. After many repetitions of the method, it was decided that, the different amino acids used in the media were not of sufficient purity. Minute traces of contaminating amino acids must have been present which were sufficient to give irregular results. Incorrect values were therefore being obtained.

APPENDIX TO SECTION 3.31. of CHAPTER 3

APPENDIX TABLE A3.31.1 Assay medium for Kelly's microbiological determination of methionine

The assay medium was prepared from two stock solutions - the basal medium and the amino acid supplement. The stock solutions were stored at 20°C. When required for use, the stock solutions were thawed and the amino acid supplement warmed to dissolve the precipitate. They were then combined in the proportion of two volumes basal medium to one volume amino acid supplement.

BASAL MEDIUM

Glucose	12g.
K_2HPO_4	18g.
Citric acid	0.5g.
Sodium acetate, $3H_2O$	2.5g.
+Tween 80	1ml.
≠Solution of mineral salts	10ml.
Adenine	5mg.
Guanine	5mg.
Uracil	5mg.
Xanthine	5mg.
Thiamine	2mg.
Pyridoxal ethylacetal hydrochloride	2mg.
Riboflavine	2mg.
Nicotinic acid	2mg.
Calcium pantothenate	2mg.
p-Amino benzoic acid	2mg.
Folic acid	0.2mg.
Biotin	10µg.
Ascorbic acid	0.5g.
Vitamin B12	2µg.

pH adjusted to 7.2 and water added to 200ml.

Amino acid sullpement.

L-glutamic acid	1g.
L-leucine	0.5g.
*L-Isoleucine	0.5g.
L-valine	0.5g.
L-Lysine hydrochloride	0.5g.
L-Alanine	0.5g.
L-Aspartic acid	0.5g.
L-Arginine hydrochloride	0.2g.
Glycine	0.2g.
øL-cystine	0.2g.
L-serine	0.2g.
L-Tyrosine	0.2g.
L-Proline	0.2g.
L-Histidine hydrochloride	0.2g.
L-Phenylalanine	0.2g.
L-Threonine	0.2g.
L-Tryptophan	0.2g.

pH adjusted with N-KOH to 7.2 and water added to 250ml.

*Allo free

øFirst dissolved separately in 10ml. boiling water by addition of HCl

+Polyoxyethylene sorbitan mono-oleate

≠Contained :

$MgCl_2,6H_2O$	20g.	$MnSO_4,4H_2O$	0.25g.
$CaCl_2$	5g.	$CoCl_2,6H_2O$	0.25g.
$FeCl_3,6H2O$	0.5g.	$CuSO4,5H_2O$	0.25g.
$ZnSO_4,7H_2O$	0.5g.	$UOSO_4$	0.25g.
$Na_2\ MoO_4$	0.25g.		

All dissolved in 1 litre of distilled water with the addition of $N\text{-}H_2SO_4$ to clear.

APPENDIX TABLE A3.31.2 Organism maintenance and inocula

Streptococcus zymogenes NCDO 592 was obtained from the National Culture of Dairy Organisms of the National Institute for Research in Dairying. The stock culture was maintained by monthly transfer from the stab culture (on casein glutamate agar medium) to the transfer medium. After incubation overnight at 37°C this culture was used to inoculate a fresh stab culture. After incubation overnight at 37°C it was stored at 4°C.

To prepare inoculum for the assay, a subculture was made from a freshly grown transfer medium culture to a tube of inoculum medium, and incubated overnight at 37°C. Subsequent inocula were then prepared by daily transfer from the inoculum culture.

The different media used are listed below

Casein-glutamate agar stabs

Basal medium	20ml.
Casein	150mg.
Sodium glutamate	15mg.
Agar	1.5g.
Distilled water to	100ml.

Steam to dissolve the agar, and dispense in 10ml. volumes. Autoclave at 115°C for 15 mins.

Transfer medium

Basal medium	20ml.
Tryptone (Oxoid Ltd.)	200mg.
Distilled water to	100ml.

Dispense in 10 ml. volumes. Autoclave at 115°C for 15 mins.

Inoculum medium

Basal medium	20ml.
Casein	150mg.
Sodium glutamate	15mg.
Distilled water to	100ml.

Dispense in 10ml. volumes. Autoclave at 115°C for 15 mins.

APPENDIX TO CHAPTER 4

APPENDIX TO SECTION 4.3 OF CHAPTER 4

Appendix Table A4.3.1 Characters scored on the white haricot bean collection at Kabanyolo during the second rains of 1970

Kabanyolo No.	Tengeru No.	Variety	Germination out of 42	Growth habit	Rust 0-5	Total seed yield g.	100 seed wt. g.	Seed size mm.	Yield Kg/hec.
1	1	Antioquia	39	IV	0	306	27.0	8	1012
2	2	Cundin a Marca 12	42	IV	0	491	24.0	7	1623
3	3	Cundin a Marca 64	33	IV	5	273	22.8	7	902
4	4	Bolivia 5	38	IV	5	203	19.0	6	671
5	7	Ecuador 16	40	IV	4	515	16.0	7	1703
6	8	Ecuador 66	41	IV	0	501	22.0	9	1657
7	9	Ecuador 68	41	IV	0	491	19.0	6	1623
8	10	Ecuador 184	35	IV	1	493	21.0	8	1630
9	13	Estados Unidos 113	40	IV	5	484	15.0	7	1601
10	14	Mexico 119	43*	IV	0	751	25.0	8	2483
11	15	Mexico 137	36	IV	0	555	23.3	6	1835
12	16	Mexico 142	32	IV	1	417	17.0	6	1379
13	17	Mexico 162	42	IV	5	496	18.0	7	1640
14	18	Mexico 184	41	IV	0	529	22.0	8	1750
15	19	Mexico.200	38	IV	0	528	20.0	7	1746
16	21	Mexico 483	38	IV	0	343	22.0	7	1134
17	24	10064-PM 9PM-6PM	41	D	5	227	15.0	8	750
18	25	Estados Unidos 112	37	IV	5	151	17.0	5	499
19	27	O.S.	42	IV	5	198	19.3	6	655
20	28	Idaho	40	IV	5	284	18.0	8	939
21	29	Chilean	38	IV	5	136	19.0	7	449
22	30	Bombost	39	IV	5	97	17.0	6	321
23	31	Local	38	IV	5	221	23.0	7	731
24	32	Michigan	29	IV	5		14.7	6	
25	33	Unknown	39	IV	5	169	17.0	5	559
26	35	Unknown	41	IV	5	129	17.0	6	427
27	36	Sanilac	35	D	5	76	13.0	5	251
28	37	Ethiopian No. 5	40	IV	5	326	21.6	7	1078

Appendix Table A4.3.1 (Contd.)

Kabanyolo No.	Tengeru No.	Variety	Germination out of 42	Growth habit	Rust 0-5	Total seed yield g.	100 seed wt. g.	Seed size mm.	Yield Kg./hec.
29	39	U.S. Pea Bean No.3	40	IV	5	170	19.0	6	563
30	40	Ohtenashi	30	IV	5	134	21.0	6	443
31	41	U.S. Pea Bean No. 1	31	D	5	128	17.0	6	423
32	42	Ethiopian No. 2	39	IV	5	325	19.0	6	1075
33	43	Chilean A	32	IV	5	117	16.0	6	387
34	44	Chilean B	41	IV	5	80	15.5	6	265
35	45	Chilean C	30	IV	5	30	14.0	6	100
36	46	Ethiopian NA	13	IV	5	41	17.0	6	136
37	47	Ethiopia	15	IV	5	18	-	7	59
38	48	Ethiopia	38	IV	5	383	17.0	7	1267
39	50	Guy	39	IV	5	120	17.0	5	397
40	51	Contessa	37	IV	5	125	17.6	5	414
41	52	Ethiopian No. 1	40	D	5	433	23.0	8	1432
42	53	Bonita	39	D	5	258	20.0	6	853
43	54	Criolla	42	IV	5	382	20.0	7	1263
44	55	Michigan	25	IV	5	34	-	7	112
45	57	Sanilac	39	D	5	295	19.0	5	975
46	58	Seaway	37	D	5	91	12.0	4	300
47	59	Michelite	29	IV	5	119	15.0	5	393
48	60	Rice Pearl	46*	IV	5	531	15.2	6	1755
49	67	GN US 1140	41	IV	5	291	21.0	8	962
50	68	Small white UI 74	40	IV	5	266	17.0	7	880
51	69	Calif. small white	40	IV	5	369	16.0	6	1220
52	70	Calif. small white	38	IV	5	393	15.0	6	1299
53	72	Small white FM 51	40	IV	3	311	15.0	7	1028
54	73	Small white FM 52	38	IV	3	475	15.0	6	1570
55	74	Small white FM 53	40	IV	3	493	14.0	6	1630
56	75		42	IV	5	316	20.2	9	1045

Appendix Table A4.3.1 (Contd.)

Kabanyolo No.	Tengeru No.	Variety	Germination out of 42	Growth habit	Rust 0-5	Total seed yield g.	100 seed wt. g.	Seed size mm.	Yield Kg./hec.
57	76		40	IV	4	516	23.6	6	1706
58	77		37	IV	1	530	20.0	7	1753
59	78		41	IV	3	413	17.0	9	1365
60	79		40	IV	5	428	29.1	10	1416
61	80		36	IV	2	481	24.0	8	1591
62	81		38	IV	5	397	19.0	8	1313
63	82		39	IV	3	455	15.8	7	1504
64	83		40	IV	5	353	25.5	8	1167
65	84		48*	IV	5	286	17.5	5	946
66	85		39	IV	1	515	26.3	8	1703
67	86		42	IV	0	395	24.0	7	1306
68	87		41	IV	5	323	19.2	7	1068
69	88		39	IV	5	388	23.0	10	1283
70	89		40	IV	3	394	25.0	8	1303
71	90		15	IV	4	212	15.9	7	701
72	92		44*	IV	3	-	-	-	-
73	93		39	IV	5	393	16.2	7	1299
74	94		42	IV	5	497	16.0	7	1643
75	95		36	IV	5	558	24.4	6	1845
76	96		48*	IV	5	518	22.6	7	1713
77	97		31	IV	5	449	21.1	9	1485
78	98		32	IV	5	313	20.0	9	1035
79	99		33	IV	5	310	18.5	7	1026
80	100		34	IV	5	568	17.3	6	1879
81		Sanilac	39	D	5	121	14.4	6	398
82		Seaway	34	D	5	265	17.8	7	884
83		Michelite	39	IV	5	120	16.0	6	398

Appendix Table A4.3.1.(contd.)

Kabanyolo No.	Tengeru No.	Variety	Germination out of 42	Growth habit	Rust 0-5	Total seed yield g.	100 seed wt. g.	Seed size mm.	Yield Kg./hec.
84		0754	41	IV	5	259	19.0	6	856
85		1509	31	D	2	471	20.0	7	1557
86		1210	39	IV	2	365	20.3	6	1207
87		1488	35	D	5	195	18.0	8	644
88		1058	42	D	4	522	18.0	8	1726
89		1046	33	D	1	513	19.7	8	1696
90		1073	42	IV	5	321	21.0	6	1061
91		Predome nain	40	IV	5	135	13.0	5	446
92		Pronel	35	D	2	430	25.7	12	1416
93		Ocop 9x	33	D	3	173	24.5	11	563
94		Pop 412	41	D	3	513	18.6	10	1702
95		Verdon	42	D	3	178	23.9	10	570
96		NEP 2	35	IB	0	777	18.6	7	2551
97		Cuarentino	33	IV	1	752	14.9	7	2476
98		Kabacuara	42	IB	0	446	20.0	6	1471
99		Emerson 247	36	D	5	284	26.5	9	939
100		Perry Marrow	20	IV	5	216	28.9	9	702
101		S1	36	D	5	275	23.5	9	909
102		S2	15	D	5	140	30.0	9	463
103		S3	25	D	5	284	29.0	8	939
104		S4	29	D	2	151	29.0	10	499
105		S5	33	D	5	528	25.3	10	1746
106		S6	37	IV	5	406	34.5	10	1343
107		S7	36	D	1	436	24.0	11	1442
108		S8	36	IV	0	-	-	-	-
109		S9	37	IV	5	384	22.0	10	1270
110		S10	25	D	5	204	22.0	10	675
111		S11	29	D	5	363	22.0	10	1200

Appendix Table A4.3.1 (contd.)

Kabanyolo No.	Tengeru No.	Variety	Germination out of 42	Growth habit	Rust 0-5	Total seed yield g.	100 seed wt. g.	Seed size mm.	Yield Kg./hec.
112		S12	32	D	5	317	22.0	10	1048
113		S13	33	D	5	363	26.0	10	1200
114		S14	29	D	2	188	22.0	8	622
115		S15	30	D	5	331	24.0	10	1094
116		S16	39	D	5	429	19.0	8	1419
117		S17	38	D	5	485	19.0	8	1604
118		S18	37	D	5	391	26.1	10	1292
119		S19	35	D	5	-	-	-	-
120		S20	38	D	3	280	22.0	7	926
121		S21	25	D	5	159	21.0	8	526
122		S22	37	D	5	299	19.0	9	989
123		S23	31	D	5	209	20.0	8	692
124		S24	38	D	3	280	20.0	8	926
125		S25	30	D	5	234	20.0	8	773
126		S26	37	D	5	285	25.0	10	943
127		S27	34	D	5	332	26.0	12	1097
128		S28	37	D	5	338	23.0	10	1118
129		S29	39	D	5	283	19.0	10	936
130		S30	36	D	5	304	22.0	11	1005
131		S31	39	D	5	271	23.0	11	896
132		S32	38	D	5	239	20.5	10	790
133		S33	38	D	5	285	24.0	9	943
134		S34	38	D	5	215	18.0	11	711
135		S35	38	D	5	165	16.0	8	546
136		S36	37	D	5	259	24.0	8	856
137		S37	40	D	4	332	24.0	8	1097
138		S38	37	D	5	369	21.0	10	1221
139		S39	35	D	5	440	21.0	9	1455

Appendix Table A4.3.1 (contd.)

Kabanyolo No.	Tengeru No.	Variety	Germination out of 42	Growth habit	Rust 0-5	Total seed yield g.	100 seed wt. g.	Seed size mm.	Yield Kg./hec.
140		S40	35	D	1	410	28.0	8	1356
141		S41	38	I	5	486	25.0	9	1608
142		S42	40	D	5	355	24.0	7	1174
143		S43	38	D	1	409	21.0	9	1353
144		S44	36	D	5	417	24.0	9	1379
145		S45	33	D	5	371	22.0	9	1226
146		S46	38	D	5	480	22.0	9	1587
147		S47	38	D	5	459	25.0	10	1518
148		S48	31	D	5	392	24.0	8	1296
149		S49	37	D	5	282	22.0	12	933
150		S50	29	D	5	277	37.9	12	916
151		D51	3	D	5	16	-	9	53
152		S52	29	D	5	244	37.3	12	807
153		S53	21	D	5	207	27.7	12	685
154		S54	3	D	5	18	-	12	59
155		S55	36	D	5	243	33.7	11	804
156		S56	33	D	5	224	28.3	11	741
157		S57	33	D	5	194	38.9	10	641
158		S58	36	D	5	64	26.5	9	212
159		S59	16	D	5	116	28.6	11	383
150		S60	19	D	5	114	30.2	10	377
161		S61	14	D	5	61	23.3	10	202
162		S62	14	D	5	173	28.6	11	572
163		S63	12	D	5	135	31.3	10	446
164		S64	16	D	5	79	25.5	11	261
165		S65	8	IV	5	196	44.3	10	648
166		S66	22	D	5	191	36.3	13	631
167		S67	6	D	5	77	30.9	12	56
168		S68	16	D	5	179	32.4	11	592

Appendix Table A4.3.1 (contd.)

Kabanyolo No.	Tengeru No.	Variety	Germination out of 42	Growth habit	Rust 0-5	Total seed yield g.	100 seed wt. g.	Seed size mm.	Yield Kg./hec.
169		S69	-	-	-	-	-	-	-
170		S70	17	D	5	143	25.6	10	473
171		S71	20	IV	5	216	48.9	9	714
172		S72	-	-	-	-	-	-	-
173		S73	-	-	-	-	-	-	-

*indicates incorrect thinning at the initial stage of the experiment

APPENDIX TO CHAPTER 5

APPENDIX TO SECTION 5.21 of CHAPTER 5

Appendix Table A5.21.1 Characters recorded for the Variety Trial grown in the 1st Rains 1971

Rep 1.

Variety	Variety Trial No.	No. of plants germinated out of 42 sown per plot	Growth habit	Rust 0-5	Maturity in days	Total plot seed yield in g.	100 seed wt. in g.	Seed size in mm.	Yield Kg./hec.
Cundin a Marca	V1	31	IV	0	89	603	24.5	7	1981
Ecuador 66	V2	21	IV	0	93	370	21.0	7	1216
Ecuador 68	V3	26	IV	0	96	543	19.0	6	1786
Ecuador 184	V4	30	IV	0	96	651	22.4	8	2138
Mexico 119	V5	30	IV	0	100	698	28.0	7	2312
Mexico 137	V6	36	IV	0	93	342	25.5	6	1125
Mexico 142	V7	23	IV	0	100	540	18.0	6	1786
Mexico 184	V8	32	IV	0	93	1086	26.0	7	3587
Mexico 200	V9	32	IV	0	93	912	27.0	7	3002
Sanilac	V10	29	D	5	79	215	16.5	5	705
Seaway	V11	36	D	5	72	173	15.0	5	571
F.M.52	V12	33	IV	3	86	609	18.0	5	1997
F.M.53	V13	30	IV	3	86	715	17.0	6	2356
Var.77	V14	32	IV	1	93	966	19.0	6	3197
Kabacuara	V15	31	IB	0	106	1032	22.0	7	3408
Cuarentino	V16	31	IV	0	96	930	29.0	7	3002
NEP 2	V17	22	IB	0	106	1122	20.4	6	3707
Pronel	V18	20	D	4	86	352	29.4	10	1156
Ocop 9X	V19	29	D	4	86	295	28.0	12	975
Pop 412	V20	29	D	1	93	528	25.0	7	1741
Verdon	V21	14	D	3	93	252	27.5	9	825
W.C. 1210	V22	35	IV	1	93	686	23.0	7	2251
W.C. 1509	V23	27	IV	1	93	745	25.0	6	2462
W.C. 1046	V24	26	IV	1	93	608	23.0	7	2011
Var. 100	V25	28	IV	2	96	873	17.0	6	2882

Appendix Table A5.21.1 (Contd.)

Rep 2.

Variety	Variety Trial No.	No. of plants germinated out of 42 sown per plot	Growth habit	Rust 0-5	Maturity in days	Total plot seed yield in g.	100 seed wt. in g.	Seed size in mm.	Yield Kg./hec.
Cundin a Marca	V1	34	IV	0	89	497	22.9	8	1637
Ecuador 66	V2	42	IV	0	96	446	21.1	8	1471
Ecuador 68	V3	37	IV	0	100	708	19.8	7	2342
Ecuador 184	V4	42	IV	0	100	665	21.2	9	2192
Mexico 119	V5	36	IV	2	100	655	24.7	7	2161
Mexico 137	V6	37	IV	0	89	658	21.0	9	2177
Mexico 142	V7	40	IV	2	89	707	17.9	7	2326
Mexico 184	V8	42	IV	0	89	698	22.3	8	2312
Mexico 200	V9	37	IV	0	89	903	21.3	9	2987
Sanilac	V10	37	D	5	73	98	13.6	6	330
Seaway	V11	36	D	5	72	117	14.1	6	390
F.M.52	V12	44*	IV	5	82	267	18.6	7	886
F.M.53	V13	40	IV	4	93	468	17.1	8	1546
Var. 77	V14	42	IV	1	85	914	20.9	9	3017
Kabacuara	V15	42	IB	0	89	1114	21.4	9	3678
Cuarentino	V16	42	IV	0	106	990	25.3	8	3272
NEP 2	V17	42	IB	0	100	1238	16.8	7	3407
Pronel	V18	37	D	1	73	110	22.2	10	360
Ocop 9X	V19	36	D	4	79	168	27.4	10	555
Pop 412	V20	37	D	2	89	481	21.4	8	1591
Verdon	V21	39	D	2	89	151	23.9	11	495
W.C. 1210	V22	36	IV	0	85	818	20.5	8	2702
W.C. 1509	V23	38	IV	1	89	739	20.7	8	2447
W.C. 1046	V24	34	IV	1	93	806	22.2	7	2657
Var. 100	V25	40	IV	3	100	663	23.4	7	2192

Appendix Table A5.21.1 (Contd.)

Rep 3.

Variety	Variety Trial No.	No. of plants germinated out of 42 sown per plot	Growth habit	Rust 0-5	Maturity in days	Total plot seed yield in g.	100 seed wt. in g.	Seed Size in mm.	Yield Kg./hec.
Cundin a Marca	V1	40	IV	0	93	487	23.6	9	1606
Ecuador 66	V2	38	IV	0	93	595	22.1	9	1952
Ecuador 68	V3	36	IV	0	82	870	20.7	7	2866
Ecuador 184	V4	42	IV	0	89	1024	23.6	7	3377
Mexico 119	V5	40	IV	1	89	791	30.2	8	2612
Mexico 137	V6	38	IV	0	85	644	28.3	7	2131
Mexico 142	V7	30	IV	2	89	481	17.3	7	1591
Mexico 184	V8	40	IV	0	89	618	25.0	9	2041
Mexico 200	V9	42	IV	0	93	1048	22.6	8	3467
Sanilac	V10	39	D	5	72	121	14.4	6	406
Seaway	V11	39	D	5	79	99	14.5	6	315
F.M.52	V12	42	IV	3	72	377	16.1	8	1245
F.M.53	V13	42	IV	3	82	427	15.7	8	1411
Var.77	V14	41	IV	0	89	545	18.1	7	1801
Kabacuara	V15	42	IB	0	89	1053	23.3	7	3482
Cuarentino	V16	33	IV	1	100	752	14.9	7	2476
NEP 2	V17	35	IB	0	95	776	18.6	7	2567
Pronel	V18	39	D	4	82	99	22.5	10	331
Ocop 9X	V19	33	D	3	79	173	24.5	11	570
Pop 412	V20	41	D	3	82	513	18.6	10	1696
Verdon	V21	42	D	3	82	178	23.9	10	585
W.C. 1210	V22	41	IV	3	89	513	18.6	10	1786
W.C. 1509	V23	38	IV	3	89	745	20.3	8	2462
W.C. 1046	V24	42	IV	0	89	505	17.1	8	1651
Var. 100	V25	38	IV	1	85	465	12.7	7	1531

*Indicates incorrect thinning at the initial stage of the experiment.

Appendix Table A5.21.2 Characters recorded for the Variety Trial grown in the 2nd Rains 1971

Rep 1.

Variety	Variety Trial No.	No. of plants germinated out of 42 sown per plot	Growth habit	Rust 0-5	Maturity in days	Total plot seed yield in g.	100 seed wt. in g.	Seed size in mm.	Yield Kg./hec.
Cundin a Marca	V1	34	IV	0	97	566	23.1	7	1876
Ecuador 66	V2	45*	IV	0	97	360	20.9	7	1186
Ecuador 68	V3	35	IV	0	80	406	17.2	7	1306
Ecuador 184	V4	37	IV	0	80	512	19.5	8	1625
Mexico 119	V5	24	IV	0	97	296	21.1	11	975
Mexico 137	V6	40	IV	0	97	638	25.3	8	2101
Mexico 142	V7	39	IV	2	97	540	15.7	7	1786
Mexico 184	V8	43*	IV	0	97	693	19.8	9	2281
Mexico 200	V9	38	IV	0	97	713	25.6	9	2356
Sanilac	V10	45*	D	5	90	52	14.9	6	165
Seaway	V11	35	D	5	90	89	19.9	6	285
F.M.52	V12	38	IV	5	90	186	15.9	7	615
F.M.53	V13	33	IV	4	88	394	17.6	8	1306
Var.77	V14	41	IV	3	88	466	17.0	6	1531
Kabacuara	V15	41	IB	0	97	775	20.9	7	2551
Cuarentino	V16	42	IV	0	97	545	28.7	8	1801
NEP 2	V17	46*	IB	0	97	629	17.6	6	2072
Pronel	V18	35	D	1	90	51	27.0	12	165
Ocop 9X	V19	43*	D	3	90	169	27.4	13	555
Pop 412	V20	42	D	1	90	382	19.7	8	1261
Verdon	V21	41	D	2	88	238	22.0	11	780
W.C. 1210	V22	41	IV	1	88	673	19.0	7	2222
W.C. 1509	V23	41	IV	1	97	394	20.0	8	1306
W.C. 1046	V24	38	IV	1	88	583	20.0	7	1921
Var.100	V25	36	IV	2	97	504	15.2	7	1666

Appendix Table A5.21.2 (Contd.)

Rep 2.

Variety	Variety Trial No.	No. of plants germinated out of 42 sown per plot	Growth habit	Rust 0-5	Maturity in days	Total plot seed yield in g.	100 seed wt. in g.	Seed size in mm.	Yield Kg./hec.
Cundin a Marca	V1	33	IV	0	70	539	26.1	7	1771
Ecuador 66	V2	31	IV	0	97	365	25.0	7	1200
Ecuador 68	V3	24	IV	0	70	687	22.0	7	2267
Ecuador 184	V4	24	IV	0	97	947	25.0	6	3002
Mexico 119	V5	38	IV	0	97	910	28.0	7	3002
Mexico 137	V6	33	IV	0	70	565	26.0	6	1855
Mexico 142	V7	28	IV	0	97	655	19.0	5	2161
Mexico 184	V8	33	IV	0	97	1015	25.0	7	3347
Mexico 200	V9	30	IV	0	97	1020	23.8	7	3362
Sanilac	V10	27	D	5	90	48	17.5	5	150
Seaway	V11	36	D	5	90	379	16.2	5	1245
F.M.52	V12	33	IV	3	90	617	18.0	6	2027
F.M.53	V13	24	IV	4	88	417	15.0	6	1365
Var.77	V14	26	IV	1	88	522	23.0	7	1726
Kabacuara	V15	26	IB	0	97	865	23.5	6	2851
Guarentino	V16	27	IV	0	97	778	27.5	8	2551
NEP 2	V17	30	IB	0	71	1292	20.3	5	4263
Pronel	V18	25	D	2	88	216	29.6	10	721
Ocop 9X	V19	22	D	5	88	381	25.0	10	1261
Pop 412	V20	18	D	1	88	238	24.0	7	780
Verdon	V21	30	D	1	88	381	28.0	8	1261
W.C. 1210	V22	37	IV	1	97	446	23.0	7	1471
W.C. 1509	V23	32	IV	2	97	476	22.0	6	1561
W.C. 1046	V24	35	IV	1	88	558	23.0	7	1846
Var. 100	V25	20	IV	3	97	618	15.0	6	2027

Appendix Table A5.21.2 (Contd.)

Rep 3.

Variety	Variety Trial No.	No. of plants germinated out of 42 sown per plot	Growth habit	Rust 0-5	Maturity in days	Total plot seed yield in g.	100 seed wt. in g.	Seed size in mm.	Yield Kg./hec.
Cundin a Marca	V1	27	IV	0	97	738	26.0	7	2431
Ecuador 66	V2	33	IV	0	97	559	24.0	6	1832
Ecuador 68	V3	26	IV	0	70	849	27.0	7	2807
Ecuador 184	V4	33	IV	0	97	753	25.0	9	2476
Mexico 119	V5	36	IV	1	97	699	27.5	9	2297
Mexico 137	V6	33	IV	0	97	869	28.0	6	2866
Mexico 142	V7	29	IV	3	97	267	14.8	6	885
Mexico 184	V8	34	IV	0	97	1025	24.0	8	3377
Mexico 200	V9	41	IV	0	97	1077	27.0	7	3542
Sanilac	V10	32	D	5	80	444	18.0	5	334
Seaway	V11	29	D	5	80	418	17.0	5	1365
F.M.52	V12	34	IV	4	80	599	18.0	6	1966
F.M.53	V13	34	IV	3	88	422	15.0	6	1381
Var. 77	V14	29	IV	0	88	538	21.0	6	1771
Kabacuara	V15	28	IB	0	97	389	23.5	6	1276
Cuarentino	V16	31	IV	1	97	365	25.0	8	1200
NEP 2	V17	28	IB	0	71	914	21.0	7	3017
Pronel	V18	31	D	4	80	382	27.0	10	1261
Ocop 9X	V19	34	D	4	88	293	22.2	12	966
Pop 412	V20	26	D	3	88	453	25.8	7	1486
Verdon	V21	27	D	3	88	319	28.0	10	1050
W.C.1210	V22	31	IV	1	88	676	23.0	6	2222
W.C. 1509	V23	32	IV	3	88	641	24.0	8	2102
W.C. 1046	V24	33	IV	0	88	760	22.0	7	2506
Var. 100	V25	32	IV	2	97	730	16.0	5	2401

*Indicates incorrect thinning at the initial stage of the experiment.

Appendix Table A5.21.3 Characters recorded for the Variety Trial grown in the 1st Rains of 1972

Rep 1.

Variety	Variety Trial No.	No. of plants germinated out of 42 sown per plot	Growth habit	Rust 0-5	Maturity in days	Total plot seed yield in g.	100 seed wt. in g.	Seed size in mm.	Yield Kg./hec.
Cundin a Marca	V1	34	IV	0	84	377	23.9	10	1246
Ecuador 66	V2	35	IV	1	97	607	21.9	9	2006
Ecuador 68	V3	34	IV	0	84	288	21.8	9	953
Ecuador 184	V4	38	IV	0	84	641	22.9	9	2130
Mexico 119	V5	32	IV	0	91	605	24.0	9	2001
Mexico 137	V6	32	IV	4	84	546	28.0	7	1806
Mexico 142	V7	36	IV	0	84	525	19.9	11	1736
Mexico 184	V8	41	IV	0	97	696	24.9	10	2301
Mexico 200	V9	41	IV	2	97	613	27.0	8	2027
Sanilac	V10	35	D	0	78	944	16.5	10	3122
Seaway	V11	34	D	5	78	265	17.8	7	877
F.M. 52	V12	36	IV	5	81	745	17.4	9	2464
F.M.53	V13	39	IV	3	81	603	16.7	9	1994
Var. 77	V14	34	IV	3	81	569	19.5	9	188
Kabacuara	V15	32	IB	0	81	791	28.0	9	2615
Cuarentino	V16	33	IV	0	91	542	28.8	11	1792
NEP 2	V17	30	IB	0	97	672	19.9	10	2222
Pronel	V18	24	D	0	91	574	27.6	11	1898
Ocop 9X	V19	34	D	2	81	536	31.9	13	1772
Pop 412	V20	29	D	1	81	540	19.0	8	1786
Verdon	V21	40	D	1	88	590	26.0	10	1951
W.C. 1210	V22	36	IV	0	81	670	20.9	9	2216
W.C. 1509	V23	36	IV	1	88	708	23.0	10	2342
W.C. 1046	V24	32	IV	1	91	606	29.5	11	2004
Var. 100	V25	38	IV	1	88	700	17.0	9	2315

Appendix Table A5.21.3 (contd.)

Rep 2.

Variety	Variety Trial No.	No. of plants germinated out of 42 sown per plot	Growth habit	Rust 0-5	Maturity in days	Total plot seed yield in g.	100 seed wt. in g.	Seed size in mm.	Yield Kg./Hec.
Cundin a Marca	V1	35	IV	0	88	625	24.9	10	2067
Ecuador 66	V2	28	IV	0	91	384	24.8	10	1270
Ecuador 68	V3	35	IV	0	97	375	22.5	9	1240
Ecuador 184	V4	35	IV	0	91	596	25.8	10	1971
Mexico 119	V5	36	IV	1	84	494	26.7	9	1633
Mexico 137	V6	31	IV	0	84	575	27.0	9	1901
Mexico 142	V7	36	IV	1	84	621	18.3	7	2054
Mexico 184	V8	34	IV	0	91	827	26.8	10	2735
Mexico 200	V9	34	IV	0	91	850	27.4	10	2811
Sanilac	V10	35	D	4	78	197	17.6	7	651
Seaway	V11	41	D	5	78	773	18.5	8	2556
F.M.52	V12	37	IV	5	82	762	17.6	9	2520
F.M. 53	V13	35	IV	3	82	491	15.7	8	1623
Var. 77	V14	37	IV	1	88	545	23.2	10	1802
Kabacuara	V15	40	IB	0	91	755	23.0	9	2496
Cuarentino	V16	28	IV	0	97	312	28.6	11	1031
NEP 2	V17	35	IB	0	97	631	21.7	9	2086
Pronel	V18	35	D	1	88	430	25.7	12	1422
Ocop 9X	V19	35	D	3	84	490	33.4	13	1621
Pop 412	V20	34	D	1	97	538	21.6	10	1779
Verdon	V21	39	D	2	88	618	20.3	12	2043
W.C. 1210	V22	40	IV	0	84	544	22.0	10	1797
W.C. 1509	V23	34	IV	1	91	773	25.8	11	2556
W.C. 1046	V24	35	IV	1	97	444	26.0	10	1468
Var. 100	V25	33	IV	2	97	309	29.0	11	1022

Appendix Table A5.21.3 (Contd.)

Rep 3.

Variety	Variety Trial No.	No. of plants germinated out of 42 sown per plot	Growth habit	Rust 0-5	Maturity in days	Total plot seed yield in g.	100 seed wt. in g.	Seed size in mm.	Yield Kg./hec.
Cundin a Marca	V1	35	IV	0	84	581	28.7	11	1921
Ecuador 66	V2	37	IV	0	88	631	23.0	9	2086
Ecuador 68	V3	34	IV	0	97	481	19.4	10	1591
Ecuador 184	V4	37	IV	0	97	793	23.6	10	2622
Mexico 119	V5	32	IV	1	91	651	30.9	10	2152
Mexico 137	V6	34	IV	0	91	571	25.6	9	1888
Mexico 142	V7	34	IV	2	97	460	19.9	8	1521
Mexico 184	V8	29	IV	0	91	595	25.9	10	1967
Mexico 200	V9	37	IV	0	91	886	24.6	10	2930
Sanilac	V10	36	D	5	78	404	16.3	7	1336
Seaway	V11	33	D	4	78	375	18.0	7	1240
F.M.52	V12	34	IV	3	81	580	16.7	9	1918
F.M.53	V13	36	IV	3	97	390	19.0	8	1290
Var. 77	V14	42	IV	0	82	875	20.6	8	2893
Kabacuara	V15	29	IB	0	85	595	21.9	9	1967
Cuarentino	V16	35	IV	1	97	821	25.3	10	2715
NEP 2	V17	30	IB	0	97	616	18.8	9	2037
Pronel	V18	35	D	3	97	500	27.0	13	1653
Ocop 9X	V19	34	D	2	88	481	29.5	13	1591
Pop 412	V20	37	D	2	82	736	20.5	10	2433
Verdon	V21	33	D	3	88	944	28.1	12	3122
W.C. 1210	V22	38	IV	1	82	766	22.6	10	2533
W.C. 1509	V23	35	IV	2	88	789	24.7	9	2610
W.C. 1046	V24	36	IV	0	88	864	22.8	10	2857
Var. 100	V25	33	IV	1	91	554	16.9	9	1831

Appendix Table A5.21.4 Characters recorded for the Variety Trial grown in the 2nd Rains of 1972

Rep 1.

Variety	Variety Trial No.	No. of plants germinated out of 42 sown per plot	Growth habit	Rust 0-5	Maturity in days	Total plot seed yield in g.	100 seed wt. in g.	Seed size in mm.	Yield Kg./hec.
Cundin a Marca	V1	35	IV	1	94	232	21.0	10	767
Ecuador 66	V2	38	IV	0	94	124	19.2	10	409
Ecuador 68	V3	36	IV	0	94	351	17.5	9	1161
Ecuador 184	V4	37	IV	2	94	368	18.8	10	1216
Mexico 119	V5	39	IV	0	94	400	24.6	10	1322
Mexico 137	V6	36	IV	1	94	542	23.2	8	1780
Mexico 142	V7	38	IV	0	94	404	15.0	8	1336
Mexico 184	V8	35	IV	0	94	260	19.5	11	860
Mexico 200	V9	23	IV	6	94	349	24.0	11	1155
Sanilac	V10	30	D	5	77	243	12.5	7	804
Seaway	V11	35	D	5	77	240	13.0	7	794
F.M. 52	V12	40	IV	3	79	229	15.4	9	758
F.M. 53	V13	39	IV	3	79	444	14.0	9	1467
Var. 77	V14	36	IV	0	91	448	16.5	9	1482
Kabacuara	V15	34	IB	0	94	670	20.6	9	2216
Guarentino	V16	35	IV	0	100	189	24.0	10	625
NEP 2	V17	36	IB	0	100	222	17.3	8	734
Pronel	V18	36	D	1	83	196	20.0	12	648
Ocop 9X	V19	35	D	1	77	270	23.0	13	893
Pop 412	V20	38	D	4	83	390	15.0	10	1290
Verdon	V21	40	D	3	83	234	19.0	12	773
W.C. 1210	V22	31	IV	0	91	440	17.2	10	1455
W.C. 1509	V23	26	IV	1	91	389	16.2	10	1285
W.C. 1046	V24	38	IV	1	91	491	13.8	10	1625
Var. 100	V25	32	IV	0	91	490	12.5	9	1619

Appendix Table A5.21.4 (Contd.)

Rep 2.

Variety	Variety Trial No.	No. of plants germinated out of 42 sown per plot	Growth habit	Rust 0-5	Maturity in days	Total plot seed yield in g.	100 seed wt. in g.	Seed size in mm.	Yield Kg./hec.
Cundin a Marca	V1	33	IV	1	94	431	23.9	10	1424
Ecuador 66	V2	34	IV	0	91	279	20.1	10	921
Ecuador 68	V3	34	IV	0	94	350	15.0	10	1158
Ecuador 184	V4	34	IV	0	100	325	20.2	10	1075
Mexico 119	V5	42	IV	0	100	641	25.0	11	2120
Mexico 37	V6	37	IV	1	94	243	21.2	8	804
Mexico 142	V7	27	IV	1	100	432	15.0	8	1429
Mexico 184	V8	30	IV	0	94	601	20.3	10	1988
Mexico 200	V9	33	IV	0	94	536	21.1	11	1772
Sanilac	V10	38	D	5	77	204	12.0	7	675
Seaway	V11	38	D	5	77	321	13.3	7	1061
F.M.52	V12	37	IV	0	77	364	15.5	9	1204
F.M.53	V13	36	IV	3	91	227	13.3	8	751
Var. 77	V14	34	IV	0	83	290	17.0	10	960
Kabacuara	V15	34	IB	0	94	779	21.7	10	2576
Cuarentino	V16	39	IV	0	100	335	19.0	11	1108
NEP 2	V17	39	IB	0	94	701	15.8	8	2318
Pronel	V18	40	D	3	83	158	18.5	11	522
Ocop 9X	V19	36	D	4	77	306	24.0	13	1012
Pop 412	V20	37	D	1	91	257	17.8	9	851
Verdon	V21	33	D	2	91	178	20.6	12	589
W.C. 1210	V22	34	IV	3	83	253	16.2	10	836
W.C. 1509	V23	36	IV	2	83	373	15.4	10	1233
W.C. 1046	W24	38	IV	0	94	350	18.0	10	1157
Var. 100	V25	34	IV	0	100	348	22.8	11	1151

Appendix Table A5.21.4 (Contd.)

Rep 3.

Variety	Variety Trial No.	No. of plants germinated out of 42 sown per plot	Growth habit	Rust 0-5	Maturity in days	Total plot seed yield in g.	100 seed wt. in g..	Seed size in mm.	Yield Kg./hec.
Cundin a Marca	V1	26	IV	2	91	442	23.5	11	1461
Ecuador 66	V2	34	IV	0	94	195	20.0	10	645
Ecuador 68	V3	35	IV	0	91	346	16.3	9	1143
Ecuador 184	V4	39	IV	0	91	299	20.9	9	989
Mexico 119	V5	33	IV	0	91	375	26.3	10	1241
Mexico 137	V6	36	IV	0	91	555	25.0	9	1835
Mexico 142	V7	34	IV	2	100	233	16.0	8	770
Mexico 184	V8	36	IV	0	94	260	19.0	10	859
Mexico 200	V9	39	IV	0	94	407	23.5	10	1346
Sanilac	V10	37	D	5	77	175	12.3	7	578
Seaway	V11	29	D	5	77	228	12.0	7	754
F.M.52	V12	32	IV	3	83	294	15.5	9	972
F.M. 53	V13	28	IV	3	91	568	15.1	9	1878
Var. 77	V14	30	IV	2	91	513	17.3	10	1696
Kabacuara	V15	26	IB	0	94	213	22.7	10	765
Cuarentino	V16	28	IV	0	100	580	19.0	10	1918
NEP 2	V17	29	IB	0	100	398	17.0	8	1316
Pronel	V18	38	D	4	91	173	22.5	12	572
Ocop 9X	V19	32	D	2	77	190	24.5	13	629
Pop 412	V20	39	D	3	91	278	17.4	10	912
Verdon	V21	39	D	3	83	286	20.8	12	947
W.C. 1210	V22	38	IV	0	91	346	16.8	10	1137
W.C. 1509	V23	30	IV	0	91	470	17.1	10	1555
W.C. 1046	V24	37	IV	3	91	391	15.0	8	1294
Var. 100	V25	34	IV	3	94	487	13.0	8	1610

APPENDIX TO SECTION 5.31 of CHAPTER 5

Appendix Table A5.31.1 Characters recorded for the Fertiliser level experiment

Rep 1.

Variety	Fertiliser level	All values are for a single plant					
		Habit	Rust	Seed size in mm.	No. of pods	No. of seeds	Seed Yield in g.
F.M.53	Control 0	IV	4	9	5	20	3.8
F.M.53	1	IV	2	8	12	71	9.8
F.M.53	2	IV	1	9	22	103	15.3
F.M.53	3	IV	1	8	13	57	9.8
F.M.53	4	IV	1	8	20	92	15.5
Mexico 142	Control 0	IV	2	7	15	70	12.3
Mexico 142	1	IV	2	7	6	13	1.6
Mexico 142	2	IV	1	7	24	111	20.2
Mexico 142	3	IV	2	7	23	102	16.9
Mexico 142	4	IV	2	7	25	130	23.5
Sanilac	Control 0	D	5	6	10	37	5.0
Sanilac	1	D	5	6	12	47	5.4
Sanilac	2	D	5	6	15	44	5.7
Sanilac	3	D	5	6	25	93	14.6
Sanilac	4	D	5	6	17	57	8.2
Kabacuara	Control 0	IB	0	8	12	39	6.7
Kabacuara	1	IB	0	9	11	46	8.3
Kabacuara	2	IB	0	9	23	81	12.7
Kabacuara	3	IB	0	9	27	117	29.3
Kabacuara	4	IB	0	9	22	11	20.8

Appendix Table A5.31.1 (Contd.)

Rep 2.

Variety	Fertiliser level	All values are for a single plant					
		Habit	Rust	Seed size in mm.	No. of pods	No. of seeds	Seed Yield in g.
F.M.53	Control 0	IV	2	8	10	47	8.2
F.M.53	1	IV	1	8	14	70	10.2
F.M.53	2	IV	1	8	26	111	15.5
F.M.53	3	IV	2	8	15	82	12.0
F.M.53	4	IV	1	8	28	115	16.0
Mexico 142	Control 0	IV	2	7	7	30	6.2
Mexico 142	1	IV	2	7	15	72	13.0
Mexico 142	2	IV	2	7	25	96	21.0
Mexico 142	3	IV	2	7	24	90	17.3
Mexico 142	4	IV	2	7	24	126	25.0
Sanilac	Control 0	D	5	6	10	39	5.6
Sanilac	1	D	5	6	12	44	5.8
Sanilac	2	D	5	6	12	46	6.2
Sanilac	3	D	5	6	20	80	12.6
Sanilac	4	D	5	6	15	64	9.8
Kabacuara	Control 0	IB	0	8	23	96	17.2
Kabacuara	1	IB	0	8	22	99	18.3
Kabacuara	2	IB	0	9	24	115	20.3
Kabacuara	3	IB	0	8	30	126	31.3
Kabacuara	4	IB	0	8	28	121	25.6

Appendix Table A5.31.1 (Contd.)

Rep 3.

Variety	Fertiliser level	Habit	Rust	Seed size in mm.	No. of pods	No. of seeds	Seed Yield in g.
F.M.53	Control 0	IV	2	8	6	40	7.3
F.M.53	1	IV	1	8	14	70	9.5
F.M.53	2	IV	1	8	23	102	15.0
F.M.53	3	IV	1	8	12	75	10.6
F.M.53	4	IV	1	8	23	101	15.8
Mexico 142	Control 0	IV	2	7	17	82	14.2
Mexico 142	1	IV	1	7	18	96	15.6
Mexico 142	2	IV	2	7	21	106	20.8
Mexico 142	3	IV	2	7	21	98	17.6
Mexico 142	4	IV	2	7	26	146	27.2
Sanilac	Control 0	D	5	6	11	38	5.6
Sanilac	1	D	5	6	10	36	5.0
Sanilac	2	D	5	6	10	38	5.9
Sanilac	3	D	5	6	28	90	15.2
Sanilac	4	D	5	6	14	45	8.6
Kabacuara	Control 0	IB	0	8	12	44	8.6
Kabacuara	1	IB	0	8	21	76	12.4
Kabacuara	2	IB	0	9	22	88	13.7
Kabacuara	3	IB	0	9	32	134	35.2
Kabacuara	4	IB	0	9	22	112	20.2

APPENDIX TO SECTION 5.32. of CHAPTER 5

Appendix Table A5.32.1

Percentage crude protein results for the variety F.M.53 when grown under different levels of fertiliser

Variety	Fertiliser Level	Rep 1	Rep 2	Rep 3
F.M.53	Control 0	18.3	22.3	21.3
F.M.53	1	23.5	23.2	21.7
F.M.53	2	23.2	23.1	23.6
F.M.53	3	23.5	20.0	23.1
F.M.53	4	21.6	17.2	21.0

Analysis of Variance Table

Source	df	SS	MS	F	
Treatments	4	24.7560	6.1890	1.8658	n.s.
Replicates	2	2.8574	1.4287	0.4307	n.s.
Error	8	26.5360	3.3170		
Total	14	54.1494			

n.s. indicates non significance

Appendix Table A5.32.2

Percentage crude protein results for the variety Mexico 142 when grown under different levels of fertiliser

Variety	Fertiliser level	Rep 1	Rep 2	Rep 3
Mexico 142	Control 0	24.2	24.7	24.2
Mexico 142	1	26.0	24.9	24.8
Mexico 142	2	25.1	25.2	25.6
Mexico 142	3	25.6	25.6	25.3
Mexico 142	4	24.9	24.8	23.0

Analysis of Variance Table

Source	df	SS	MS	F	
Treatments	4	4.0694	1.0173	3.1272	n.s.
Replicates	2	0.9374	0.4687	1.4408	n.s.
Error	8	2.6026	0.3253		
Total	14	7.6094			

n.s. indicates non significance

Appendix Table A5.32.3

Percentage crude protein results for the variety Sanilac when grown under different levels of fertiliser

Variety	Fertiliser Level	Rep 1	Rep 2	Rep 3
Sanilac	Control 0	22.7	24.8	24.8
Sanilac	1	24.2	24.2	25.6
Sanilac	2	25.0	29.7	28.8
Sanilac	3	18.9	22.7	25.5
Sanilac	4	26.3	28.6	25.0

Analysis of Variance Table

Source	df	SS	MS	F	
Treatments	4	70.3573	17.5893	7.5969**	
Replicates	2	13.964	6.982	3.0155	n.s.
Error	8	18.5227	2.3153		
Total	14	102.844			

** indicates significance at the 1% level
n.s. indicates non significance

Appendix Table A5.32.4

Percentage crude protein results for the variety Kabacuara when grown under different levels of fertiliser

Variety	Fertiliser Level	Rep 1	Rep 2	Rep 3
Kabacuara	Control 0	24.6	23.7	23.4
Kabacuara	1	24.8	23.5	23.7
Kabacuara	2	28.6	27.6	24.7
Kabacuara	3	20.6	21.3	20.2
Kabacuara	4	27.2	23.5	24.1

Analysis of Variance Table

Source	df	SS	MS	F
Treatments	4	61.5666	15.3916	13.9593**
Replicates	2	9.6520	4.8260	4.3769*
Error	8	8.8214	1.1026	
Total	14	80.04		

*indicates significance at the 5% level

** indicates significance at the 1% level

APPENDIX TO SECTION 5.34 of CHAPTER 5

Appendix Table A5.34.1 Methionine results (mg.available methionine/g. dry bean) for the variety F.M.53 when grown under different levels of fertiliser (each value is a mean of duplicate samples)

Variety	Fertiliser level	Rep 1	Rep 2	Rep 3
F.M.53	Control 0	0.63	0.66	0.67
F.M.53	1	0.59	0.61	0.66
F.M.53	2	0.62	0.59	0.59
F.M.53	3	0.67	0.64	0.59
F.M.53	4	0.56	0.64	0.64

Analysis of Variance Table

Source	df	SS	MS	F	
Treatments	4	0.005	0.001	0.50	n.s.
Replicates	2	0.001	0.001	0.50	n.s.
Error	8	0.012	0.002		
Total	14	0.018			

n.s. indicates non significance

Appendix Table A5.34.2 Methionine results (mg. available methionine/g. dry bean) for the variety Mexico 142 when grown under different levels of fertiliser (each value is a mean of duplicate samples)

Variety	Fertiliser Level	Rep 1	Rep 2	Rep 3
Mexico 142	Control 0	0.64	0.66	0.69
Mexico 142	1	0.64	0.66	0.63
Mexico 142	2	0.66	0.70	0.64
Mexico 142	3	0.66	0.67	0.72
Mexico 142	4	0.66	0.70	0.63

Analysis of Variance Table

Source	df	SS	MS	F	
Treatments	4	0.003	0.001	1.00	n.s.
Replicates	2	0.002	0.001	1.00	n.s.
Error	8	0.009	0.001		
Total	14	0.014			

n.s. indicates non significance

Appendix Table A5.34.3 Methionine results (mg. available methionine/g. dry bean) for the variety Sanilac when grown under different levels of fertiliser (each value is a mean of duplicate samples)

Variety	Fertiliser Level	Rep 1	Rep 2	Rep 3
Sanilac	Control 0	1.82	1.88	1.74
Sanilac	1	1.81	1.77	1.82
Sanilac	2	1.80	1.83	1.75
Sanilac	3	1.83	1.78	1.70
Sanilac	4	1.87	1.75	1.80

Analysis of Variance Table

Source	df	SS	MS	F	
Treatments	4	0.003	0.001	0.333	n.s.
Replicates	2	0.011	0.006	2.000	n.s.
Error	8	0.020	0.003		
Total	14	0.034			

n.s. indicates non significance

Appendix Table A5.34.4 Methionine results (mg. available methionine/g. dry bean) for the variety Kabacuara when grown under different levels of fertiliser (each value is a mean of duplicate samples)

Variety	Fertiliser Level	Rep 1	Rep 2	Rep 3
Kabacuara	Control 0	1.72	1.85	1.82
Kabacuara	1	1.80	1.77	1.74
Kabacuara	2	1.67	1.70	1.74
Kabacuara	3	1.82	1.70	1.77
Kabacuara	4	1.83	1.68	1.83

Analysis of Variance Table

Source	df	SS	MS	F	
Treatments	4	0.015	0.004	1.00	n.s.
Replicates	2	0.004	0.002	0.50	n.s.
Error	8	0.032	0.004		
Total	14	0.051			

n.s. indicates non significance